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CTFR **Steroide**; Dicetone; Corticosteroide; Oxydation; Epoxydation; Fluor Compose organique; Acetalisation; Pregnadiene-4,17(20)dione-3,16; Pregnene-'4'dione-3,20(acetoxy-'21' hydroxy-'17.alpha.' methyl-'16.alpha.'); Pregnadiene-'1,4'dione-3,20(fluoro-'9.alpha.' methyl-'16.alpha.' trihydroxy-'11.beta.,17.alpha.,21')

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HIGHER ISOPRENOIDS — XIX[a]

GUGGULSTERONES TO DEXAMETHASONE

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Abstract — Guggulsterones {*E*- and *Z*-pregna-4,17(20)-diene-3,16-dione}, which constitute some 2% of the gum-resin from *Commiphora mukul*, have obvious functionality suitable for elaboration into clinically useful steroidal drugs. In an effort to demonstrate this potential, guggulsterone mixture has been converted into 21-acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione, which has been earlier transformed into the well-known corticosteroid dexamethasone (9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione)

Bulk of therapeutically useful steroids continue to be made from natural steroidal raw materials such as diosgenin, sterols and bile acids. Of these, diosgenin had till recently occupied a place of preeminence, but has become less important with the commercialization of fermentation of cholesterol and sitosterol to androstenedione and androstadienedione. However, 16-dehydropregnenolone (1), obtainable from diosgenin by Marker degradation, has some obvious advantages for the manufacture of C_{21} steroids, such as corticosteroids and continues to be exploited towards that end.[1-3] The discovery of occurrence of pregnane derivatives, *Z*- and *E*-pregna-4,17(20)-diene-3,16-diones (*Z*- and *E*-guggulsterones; 2, 3)[4], to the extent of some 2% in the gum-resin from *Commiphora mukul* (Hook. ex Stocks) Engl., prompted us to explore this material as a possible useful steroidal raw material, especially since this gum resin is a commercial product in India. The tree *Commiphora mukul* grows wild in the semi-arid regions of India and the annual production of its gum-resin (*guggulu* in Sanskrit) has been estimated (1975) at around 400 tonnes.[5]

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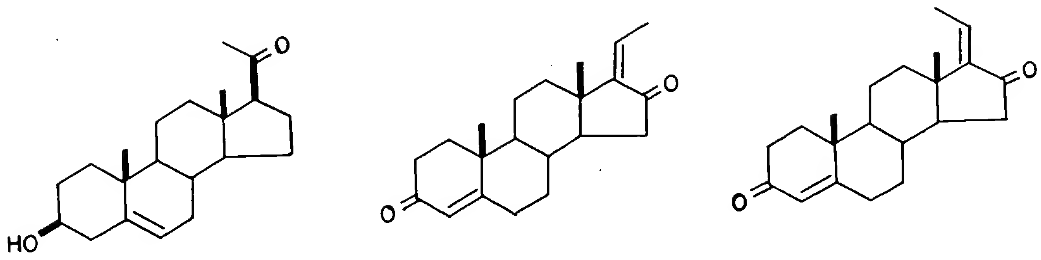
[a] Part XVIII, Indian J. Chem. 22B, 989 (1983)
[b] MRC Communication No. 58
[c] Abstracted from the Ph.D. Theses of S. Swaminathan (1985) and R.K. Bakshi (1983), M.S. University, Baroda



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Structure of guggulsterone(s) is well-suited for elaboration into a host of C_{21} steroidal drugs. However, its oxygen function at C-16 confers on it a special advantage for transformation into compounds with additional functionality at C-16. In an effort to demonstrate this potential, guggulsterone mixture (*Z* and *E*) has been elaborated into 21-acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione (4). This compound has been earlier[6] converted into its 1-dehydro derivative (5), an intermediate in the Oliveto synthesis[7] of dexamethasone (6; 9α-fluoro-16α-methyl-11β,17α,21-trihydroxypregna-1,4-diene-3,20-dione). Dexamethasone is a reputed glucocorticoid.[8]

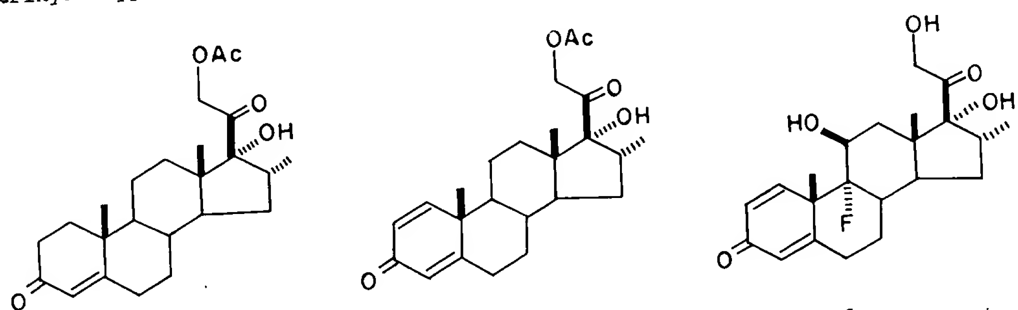

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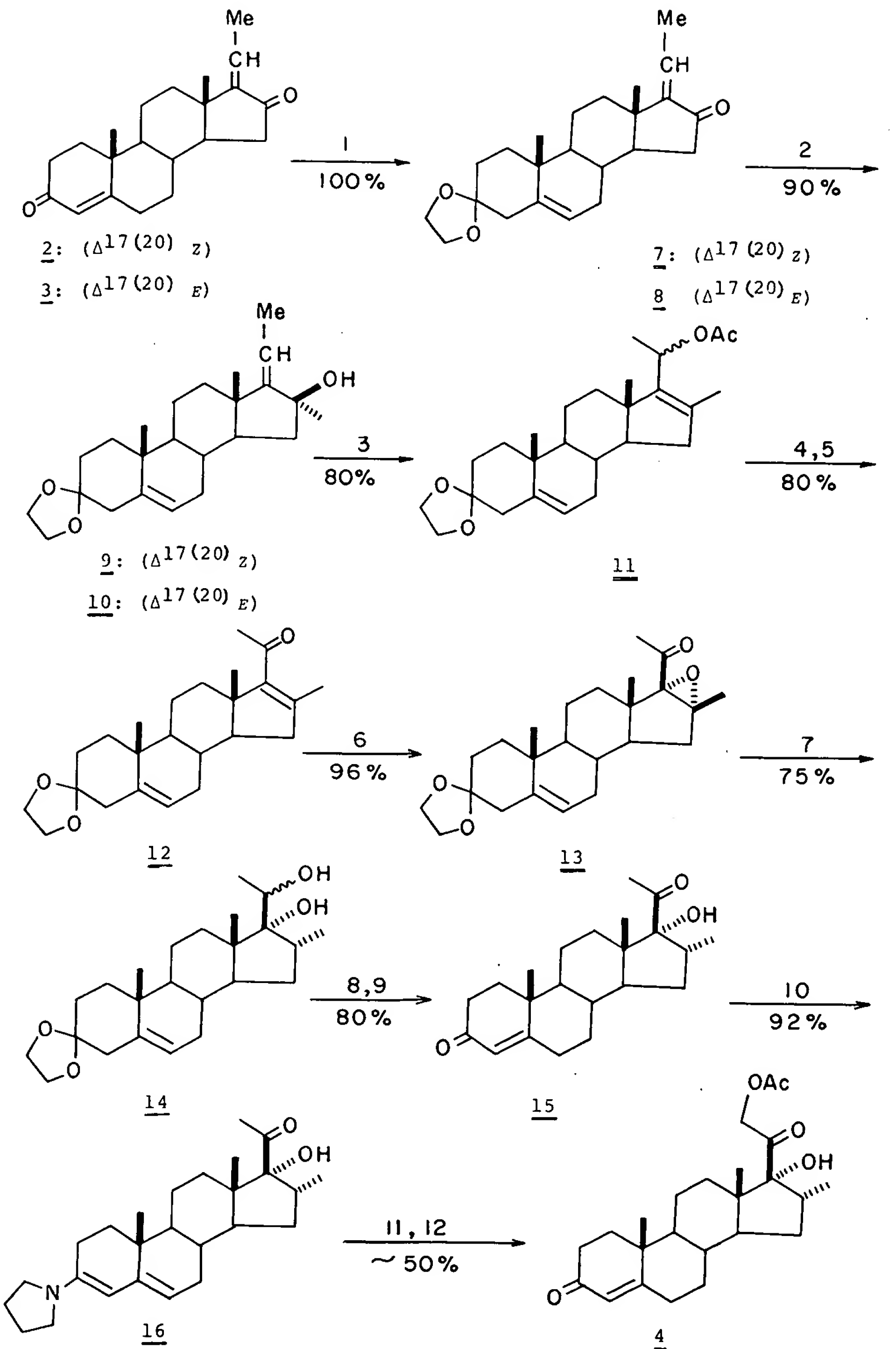
Fig. 1 depicts the route finally developed, after considerable experimentation, for the targeted conversion : guggulsterones (2, 3) to 4. The overall yield is ~16%.

The first step called for selective protection of the C-3 carbonyl, so that C-16 carbonyl could then be manipulated in the desired manner. Though, there was little information available in the literature regarding the relative reactivities of the 3-keto-4-ene and 16-keto-17(20)-ene systems, the C-3 carbonyl, being less hindered, was expected to be more reactive.[9] Keeping in view the subsequent requirements, protection through ketalization appeared most appropriate, and transketalization being more selective,[10] offered the best possibility. In practice, reaction of guggulsterones with 2-ethyl-2-methyl-1,3-dioxolan in presence of *p*-toluenesulfonic acid (*p*-TSA), under suitable conditions, furnished the required dioxolan mixture (7, 8) in 84% yield (~100% based on unrecovered starting material).



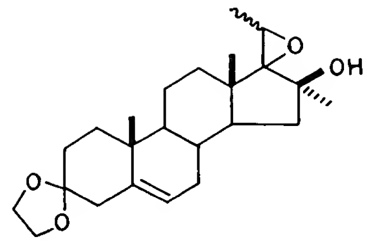
Reagents : 1. 2-Ethyl-2-methyl-1,3-dioxolan, *p*-TSA
2. MeLi, TMEDA
3. Ac_2O, AcOH, CH_2Cl_2, *p*-TSA
4. 5% KOH-EtOH
5. Pyridinium chlorochromate
6. H_2O_2 aq, NaOH aq
7. LAH, THF
8. DMSO, $(COCl)_2$
9. MeCOMe, *p*-TSA
10. Pyrrolidine, *i*-PrOH
11. EtOH-HCl; Br_2-EtOH
12. KOAc, MeCOMe

Fig. 1. Transformation of guggulsterones into 21-acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione

It was found that during this reaction isomerization of $\Delta^{17(20)}$ also occurred. Thus, though the starting guggulsterone mixture consisted of approx. 3:1 of 2 and 3, the product contained 7 and 8 in the ratio of ~1:1. This isomerization, which is unexceptional, was confirmed by carrying out the reaction with pure *Z*-guggulsterone (2), when again ~1:1 mixture of 7 and 8 were obtained. This mixture of dioxolanes (7,8) was used as such in the next step, though for characterization purposes pure 7 and 8 were obtained by chromatography. Identification of 7 and 8 is based on their ^{1}H-NMR spectra: because of the deshielding effect[11] of the C-16 carbonyl the *syn*-substituent at C-20 would suffer a down-field shift; thus the compound with δC-20 Me at 2.05 ppm (other isomer, 1.86 ppm) and δC-20 H at 5.68 ppm (other isomer, 6.45 ppm) has been assigned the *Z*-configuration (7).

The next step was aimed at introduction of C-16 methyl through an organometallic reagent. Configuration of methyl at C-16 is unimportant as chirality at this centre would be lost in the next step. Preliminary experiments with MeMgI in ether or tetrahydrofuran (THF) showed that the reaction was sluggish and remained incomplete even after refluxing for 24 hr: yield of 9/10 being 22% and 40% respectively for ether and THF. As expected, methyllithium proved more reactive. Its reaction with *E*-ketal (8) was complete in 4 hr, though *Z*-ketal (7), because of increased steric hindrance, gave the required alcohol 9 in 75% yield after a reaction period of 15 hr. However, the addition of methyllithium to both 7 and 8 could be accelerated in presence of tetramethylethylenediamine (TMEDA), which is known to activate alkyllithium reagents.[12] Thus, the reaction of 7/8 with methyllithium under these conditions was essentially complete in 3 hr to furnish 9/10 in 90% yield. The 16α-configuration assigned to the incoming methyl group is based on mechanistic considerations and analogies from literature.[13]

Suitable allylic tertiary alcohols have been converted by chromium (VI) oxidants to αβ-olefinic aldehydes (ketones) through *in situ* 1,3-transposition followed by oxidation.[14-17] However, in an effort to convert 9/10 directly into 12 by such an oxidation, the expected reaction did not occur with Jones reagent[15] or pyridinium chlorochromate[16b] or 3,5-dimethylpyrazole-CrO_3 complex.[17] The last two reagents furnished complex, difficult-to-separate mixtures, while from Jones oxidation reaction a product (in ~35% yield) formulated as a mixture of 17,20-epoxy compounds (17), could only be obtained. However, the desired conversion could be effected by a two-step process. The alcohol mixture (9, 10), on exposure to acetic anhydride, acetic acid in CH_2Cl_2 in presence of *p*-TSA,[18] furnished the rearranged acetate 11 (along with some deketalized product). Saponification of 11 followed by oxidation with pyridinium chlorochromate gave the required ketone 12. Another sample of 12 was prepared by ketalization of the known 16-methylpregna-4,16-diene-3,20-dione (18).[19] The two preparations were identical in all respects.



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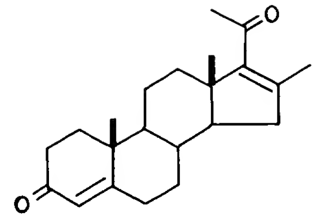

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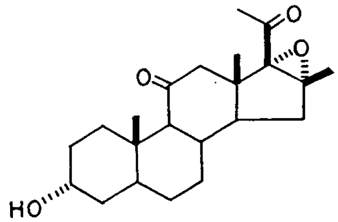
The next objective called for a sequence of reactions permitting stereospecific introduction of 17α-OH function while ensuring α-configuration for the C-16 methyl. Epoxidation of 12 followed by reductive oxirane ring-cleavage appeared as the logical choice of reactions. Epoxidation with alkaline H_2O_2[20,21] should attack only Δ^{16} and since the attack would preferentially proceed from the less-hindered α-face of the steroid molecule,[21,22] the product should be 13. In practice, oxidation with alkaline H_2O_2 in CH_2Cl_2-EtOH furnished 16,17-epoxide (in 96% yield) which, in view of the above remarks, was formulated as 13. The course of the reaction is best followed by IR spectrophotometry (change of $\nu^{C=O}$ from 1658 cm^{-1} to 1695 cm^{-1}). In spite of a negative report[21] on the attempted ring-opening of the oxirane in the closely related compound 19 with LAH, we decided to investigate this reaction with our epoxide 13. LAH reduction in refluxing ether resulted in reduction of C-20 carbonyl only. However, ultimately it was found that when this reaction was carried out in refluxing THF and excess of LAH, the desired reaction occurred to give a diol in a yield of 75%. It is obvious that the hydride attack must have occurred from the β-face, but the question of regiospecificity remains. In principle, the hydride attack can take place at either of the two positions C-16, C-17, though C-16 position being less hindered, was expected to be preferred. In fact, LAH reduction of 16,17-α-epoxypregnanes is known[23] to give 17-α-ols. Furthermore, it is reasonable to assume that the C-20 carbonyl is first rapidly reduced and the oxirane cleavage of the resulting epoxyalcohol then occurs. It has been postulated[24] that regioselectivity in the ring-opening in certain epoxyalcohols is best explained by invoking a six-membered transition state. Thus, in the present case such a situation (cf 20) would lead to delivery of hydride at C-16 leading specifically to the diol 14. Stereochemistry at C-20 is unimportant, as it will be destroyed at the next stage. Structure 14 is supported by its electron impact-induced fragmentation; it undergoes cleavage typical of α-glycols,[4,25] as shown in 21. Its ^1H-NMR spectrum displays a 3H doublet at δ0.95 ppm (J = 7 Hz) expected of secondary methyl at C-16.



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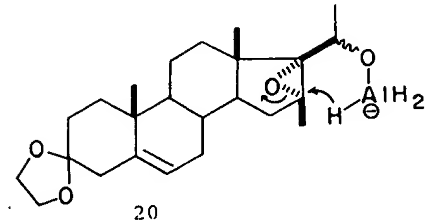

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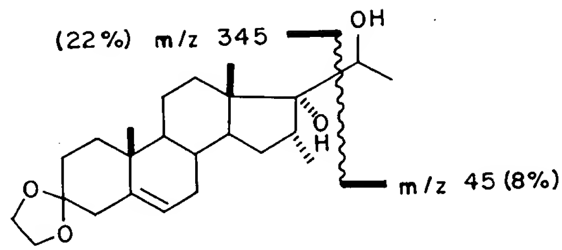

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Oxidation of 14 with DMSO-oxalyl chloride[26] furnished in over 80% yield the corresponding C-20 ketone. The latter on ketal cleavage gave the hydroxydiketone 15. This compound has been earlier prepared[6] from 3β,17α-dihydroxy-16α-methylpregn-5-en-20-one.

Final objective, namely hydroxylation of 15 at C-21, required a lot of exploratory studies. Procedure finally worked out successfully is depicted in Fig. 1 and exploits the fact that the 4-en-3-one system in a 17α-hydroxy-20-keto-pregnane can be preferentially protected through its enamine with pyrrolidine and C-21 brominated under the usual acidic conditions.[27,28] Thus, the dienamine 16[28] obtained in 92% yield was converted into its eniminium chloride and brominated, and the resulting product hydrolysed (20% $KHCO_3$aq) to get 21-bromo-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione. The latter compound, without isolation was treated with KOAc in refluxing acetone to get the known[6] 21-acetoxy-17α-hydroxy-16α-methyl-pregn-4-ene-3,20-dione (4).

EXPERIMENTAL

All m.ps are uncorrected. Pet. ether refers to light petroleum fraction b.p. 60-80°. Tetrahydrofuran was distilled from sodium benzophenone ketyl immediately prior to use.[29] Dimethyl sulphoxide was distilled from CaH_2 under reduced pressure and kept over 4A molecular sieves. Acetone was refluxed over $KMnO_4$ and then distilled from anhyd. K_2CO_3. All solvent extracts were finally washed with brine and dried (Na_2SO_4).

Silica gel for chromatography (-100, + 200 mesh) was washed with hot water till sulphate-free, dried and activated at 125-130° for 6 hr and standardized.[30] TLC was carried out on silica gel layers (0.25 mm) containing 15% gypsum and activated at 110-115° (2 hr); visualization: 1% vanillin in 30% H_3PO_4 aq spray followed by heating ~100°/5 min. Course of all reactions and chromatographies was followed by TLC.

The following instruments were used for spectral/analytical data: Schmidt + Haensch electronic polarimeter model Polatronic 1; Perkin-Elmer model 781 Infrared Spectrophotometer; Perkin-Elmer model 402 Ultraviolet Spectrophotometer; Perkin-Elmer model R32 (90 MHz) NMR Spectrometer: Varian Mat CH7 Mass Spectrometer (70 eV, direct inlet system); Hewlett-Packard Model 185B C,H,N Analyzer. All optical rotations were measured in $CHCl_3$ soln unless stated otherwise. All ^{1}H-NMR spectra were recorded in $CDCl_3$ with TMS as internal reference; signals are reported in ppm (δ); while citing ^{1}H-NMR data, following abbreviations have been used: s (singlet), d(doublet), t(triplet), q(quartet), m(multiplet), b(broad). While summarising mass spectral data, besides the molecular ion, nine other most important ions (m/z) are reported with nine other most important ions (m/z) are reported with their relative intensities.

3,3-Ethylenedioxypregna-5,17(20)-dien-16-ones (7,8)

A mixture of *Z*- and *E*-guggulsterones (2,3; m.p. 140-170°; 900 mg), *p*-toluenesulfonic acid (*p*-TSA) (20 mg), and 2,2-ethylenedioxybutane (16 ml) was heated at 110° (bath temp.) and 2-butanone (mixed with some 2,2-ethylenedioxybutane) slowly stripped off (6 ml) during 6 hr. At the end of this period, the reaction mixture was cooled, *p*-TSA neutralized by stirring with 5% Na_2CO_3 aq (10 ml) for 15 min at 20°. The organic material was taken up in EtOAc (20 ml), washed with water, dried and freed of solvent to give a residue (980 mg) which was chromatographed over SiO_2-gel/IIA (2.5 cm x 40 cm). Elution was carried out with pet. ether containing 5% EtOAc. After rejecting 50 ml of first eluates (10 mg of material), the next 25 ml x 8 of solvent eluted 450 mg of 7. The next 25 ml x 2 of eluate gave a mixture of 7 and 8 (80 mg). This was followed by compound 8 (400 mg, 25 ml x 8). Finally, last 25 ml x 4 eluted 35 mg of guggulsterone mixture (1:1).

Z-Isomer (7). The product was recrystallized from ethyl acetate-pet ether to afford leaflets, m.p. 176-178°, $[\alpha]_D$ + 38.5°. λ_{max}^{EtOH} 245 nm, ε = 11400. IR (Nujol): 1710, 1640, 1420, 1360, 1340, 1310, 1261, 1230, 1100 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 0.94, 1.07 ppm), Me-CH=C (3H, d, 2.05 ppm, J = 7Hz), OCH_2CH_2O (4H, s, 3.89 ppm), CH=C (1H, m, 5.2-5.35 ppm; 1H, q, 5.68 ppm, J = 7 Hz). Mass: m/z 356 (M^+, 67%), 91(100%), 135(33%), 119(49%), 105(62%), 93(43%), 86(40%), 79(70%), 77(52%), 67(36%). (Found: C, 77.60; H, 8.85. $C_{23}H_{32}O_3$ requires: C, 77.49; H, 9.05%).

E-Isomer (8). Recrystallization of this material furnished leaflets, m.p. 207-208°, $[\alpha]_D$ + 45.5°. λ_{max}^{EtOH} 245 nm, ε = 13,500. IR (Nujol): 1721, 1648, 1429, 1320, 1300, 1270, 1248, 1195, 1131, 1102, 1008, 942, 865 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 1.06, 1.09 ppm), Me-CH=C (3H, d, 1.86 ppm, J = 7Hz), OCH_2CH_2O (4H, s, 3.91 ppm), CH=C (1H, m, 5.2-5.4 ppm; 1H, q, 6.45 ppm, J = 7 Hz). Mass: m/z 356 (M^+, 20%), 55 (100%), 119(19%), 105(30%), 100(94%), 93(20%), 91(46%), 86(19%), 79(32%), 77(25%). (Found: C, 77.50, H, 8.92; $C_{23}H_{32}O_3$ requires: C, 77.49; H, 9.05%).

3,3-Ethylenedioxy-16α-methylpregna-5,17(20)-dien-16-ols (9,10)

To a soln of ketals 7 and 8 (3.56 g, 0.01 mole) in dry ether (200 ml) was added TMEDA (4 ml), and a soln of MeLi in ether (0.280 g in 10 ml ether; 0.013 mol) introduced, with stirring, at room temp. (25°) under anhydrous conditions and under a blanket of N_2. The reaction mixture was stirred at room temp. for a total of 3hr, and then worked up by addition of ice-cold satd. NH_4Cl aq (35 ml) and extraction with ether. Removal of solvent gave a material (3.65 g) which was chromatographed on SiO_2-gel/II; 5% EtOAc in pet. ether eluted the required product (9 + 10, m.p. 140-145°; 3.35 g), which was used as such in the next step.

For characterization purposes pure 9 and 10 were prepared from pure 7 and 8 respectively.

Z-Isomer (9). A soln of *Z*-ketal 7 (500 mg) in anhyd. ether (40 ml) was reacted with MeLi (37 mg in 2 ml ether) as above (no TMEDA) and worked up after 15 hr stirring to get a product (530 mg) which was chromatographed over SiO_2-gel/II (1.5 cm x 30 cm). Elution was carried out with 5% EtOAc in pet. ether (10 ml cuts). First eluates (100 ml) gave unchanged starting material (105 mg). After rejecting the intercut (50 ml; 40 mg of mixture), the next 300 ml eluted 314 mg of pure 9, which was recrystallised from EtOAc-pet. ether, m.p. 149-151°, $[\alpha]_D$ + 34.4° (EtOH). IR (Nujol):

3500, 1315, 1258, 1140, 1095, 1085, 1020, 945, 865, 820, 800 cm^{-1}. ^1H-NMR: Me (3H singlets at 0.91, 1.02, 1.41 ppm), Me-CH=C (3H, d, 1.82 ppm, J = 7Hz), OCH_2CH_2O (4H, s, 3.89 ppm), CH=C (2H, m, 5.1-5.42 ppm). Mass: m/z 372 (M^+, 46%), 100(100%), 133(16%), 119(23%), 105(29%), 93(20%), 91(35%), 79(23%), 55(73%), 43(65%). (Found: C, 77.48; H, 9.90. $C_{24}H_{36}O_3$ requires: C, 77.37; H, 9.74%).

E-Isomer (10). A similar reaction of *E*-ketal 8 (450 mg) in dry ether (40 ml) with MeLi (32 mg in 2 ml ether) for 4 hr furnished a product (500 mg) which on chromatography as above gave, besides the starting ketal (10 mg), 393 mg of the desired product 10, recrystallized from EtOAc-pet.ether, m.p. 171-173°, $(\alpha)_D$ + 76.6° (EtOH). IR (Nujol): 3492, 2860, 1348, 1272, 1260, 1240, 1200, 1160, 1141, 1080, 1040, 1021, 950, 932, 878, 821, 804 cm^{-1}. ^1H-NMR: Me (3H singlets at 1.07, 1.10, 1.31ppm), Me-CH=C (3H,d,1.74 ppm, J = 7 Hz), OCH_2CH_2O (4H, s, 3.95 ppm), CH=C (2H,m,5.3-5.7 ppm). Mass: m/z 372(M^+, 38%), 100(100%), 119(23%), 105(30%), 93(20%), 91(37%), 79(26%), 55 (85%), 43(75%), 41(28%). (Found: C, 77.38, H, 9.87; $C_{24}H_{36}O_3$ requires: C, 77.37; H,9.74%).

Oxidation of ketal alcohols (9,10) with Jones reagent: isolation of 3,3-ethylenedioxy-17,20-epoxy-16α-methylpregn-5-en-16-ols (17)

To a mixture of ketal alcohols 9 and 10 (88 mg, 0.24 mmole) in acetone (20 ml) at -15°, Jones reagent[31] (0.1 ml) was added and the reaction mixture stirred at that temp. for 5 min. Usual work up gave a product (82 mg) which was chromatographed over SiO_2-gel/II (10 g) using 5% EtOAc in pet. ether as eluant. First 50 ml eluted a product (foam, 40 mg) characterized as 17. IR (Nujol): 3520, 2850, 1319,1300, 1250, 1240, 1204, 1158, 1135, 1095, 1082, 1045, 1020, 1005, 980, 942, 902, 865, 820, 800 cm^{-1}. ^1H-NMR: Me (9H, bs, 1.0 ppm; 3H, s, 1.12 ppm), OCH_2CH_2O (4H, s, 3.92 ppm), CH=C (1H, m, 5.4 ppm). (Found: C, 74.15, H, 9.21; $C_{24}H_{36}O_4$ requires: C, 74.19; H, 9.34%).

3,3-Ethylenedioxy-16-methylpregna-5,16-diene-20-acetate (11)

To a cooled (-10°) soln of anhydrous acetic acid (15 ml), and acetic anhydride (freshly distilled over P_2O_5; 5 ml) in CH_2Cl_2 (20 ml) containing *p*-TSA (150 mg), ketal alcohol (9,10) mixture (3.0 g, 0.008 mole) was added (5 min) with stirring. After stirring for another 12-15 min, the contents were poured onto a mixture of crushed ice (~100 g) and 15% Na_2CO_3 aq (~0°, 100 ml). The organic layer was separated, the aq phase extracted with CH_2Cl_2 (50 ml x 3) and the combined extract washed with 5% Na_2CO_3 aq (50 ml x 2), water and dried. Removal of solvent furnished a product (295 mg) which was chromatographed over SiO_2-gel/II (90 g);elution was carried out with 5% EtOAc in pet. ether (25 ml cuts). After rejecting the first eluates (50 ml; 50 mg product), the next 250 ml eluted the required acetate 11 (1.82 g, m.p. 105-110°). The next 100 ml gave a mixture (75 mg), while the last 250 ml yielded (750 mg) essentially deketalized product corresponding to acetate 11.

The required acetate (11; apparently 1:1 mix. of C-21 acetates) showed the following spectral characteristics. IR (Nujol): 1736, 1240, 1202, 1100, 1062, 1049, 1020, 950, 908, 860 cm^{-1}. ^1H-NMR: Me (3H, two singlets at 0.88 and 0.92 ppm; 3H, s, 1.08 ppm; 3H, d, 1.38 ppm, J = 7Hz), Me-C=C (3H, two singlets at 1.74 and 1.76 ppm), $OCOCH_3$ (3H, s, 2.02 ppm), OCH_2CH_2O (4H, s, 3.94 ppm), CH=C (1H, m, 5.27-5.44 ppm), CHOAc (1H, m, 5.45-5.88 ppm). Mass: m/z 413 (M^+-1, 12%), 55(100%), 119(37%), 105(66%), 100(94%), 91(72%), 79(54%), 77(40%), 67(24%), 45(49%). (Found: C, 75.41; H, 9.20; $C_{26}H_{38}O_4$ requires: C, 75.32; H, 9.24%).

The deketalized fraction was best again ketalized in the usual manner to get more of 11.

3,3-Ethylenedioxy-16-methylpregna-5,16-dien-20-ol

The above acetate (11; 750 mg, 1.8 mmole) and 5% KOH-EtOH (50 ml) were refluxed (4 hr) and worked up as usual to get the deacetylated derivative (730 mg, m.p. 154-157°), which was used as such for the next step. A small sample was crystallised from EtOAc-pet.ether, m.p. 157-159°. IR (Nujol): 3498, 1452, 1422, 1318, 1300, 1265, 1232, 1205, 1140, 1100, 1020, 995, 952, 870, 812 cm^{-1}. ^1H-NMR: Me (3H, two singlets at 0.89 and 0.91 ppm; 3H, s, 1.05; 3H, d, 1.35 ppm, J = 7Hz), Me-C=C (3H, two singlets at 1.72 and 1.77 ppm), OCH_2CH_2O (4H, s, 3.92 ppm), CHOH (1H, m, 4.40-4.80 ppm), CH=C (1H, m, 5.20, 5.45 ppm). Mass: m/z 372(M^+, 74%), 100(100%), 357(47%), 327(51%), 119(33%), 105(46%), 93(39%), 91(50%), 79(42%), 55(66%).

3,3-Ethylenedioxy-16-methylpregna-5,16-dien-20-one (12)

(a) Above alcohol (136 mg, 0.365 mmole) in CH_2Cl_2 (15 ml) was treated with pyridinium chlorochromate[32] (136 mg) at room temp. (~30°) for 1 hr and worked up with ether in the usual manner. Solvent removal gave a product (197 mg), which was filtered through a bed of SiO_2-gel/II (10 g) using 5% EtOAc in pet.ether as solvent. This material, thus obtained, was recrystallized from EtOAc to give a white crystalline solid, m.p. 214-216°, $[\alpha]_D$ + 55.1°. IR ($CHCl_3$): 1662, 1600, 1450, 1428, 1375, 1360, 1330, 1220, 1125, 1100, 1020, 950, 860 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 0.98 and 1.06 ppm), Me-C=C (3H, s, 2.02 ppm), MeCO (3H, s, 2.22 ppm), OCH_2CH_2O (4H, s, 3.87 ppm), CH=C (1H, m, 5.15-5.37 ppm). Mass: m/z 370(M^+, 19%), 43(100%), 119(16%), 105(27%), 100(50%), 93(15%), 91(34%), 79(20%), 55(66%), 41(16%). (Found: C, 77.79; H,9.22; $C_{24}H_{34}O_3$ requires: C, 77.80; H, 9.25%).

(b) From 16-methylpregna-4,16-diene-3,20-dione. A mixture of this dione[19] (3.0 g, 9.2 mmole), p-TSA (110 mg) and 2,2-ethylenedioxybutane (60 ml) was heated (110°, bath temp.) with distillation of 2-butanone exactly as already described for 7, 8.. The product (3.3 g), thus obtained, was chromatographed over Al_2O_3/II (2 cm x 67.5 cm), while eluting with 5% EtOAc in pet.ether (100 ml cuts). After rejecting the first 300 ml of eluate (585 mg of product), the next 700 ml eluted the required product (1.73 g, m.p. 213-215°), which was recrystallised from EtOAc, m.p. 214-216°, identical in all respects (m.p., mixed m.p., IR, ^{1}H-NMR) with the product described under (a). Last 500 ml of eluate gave 550 mg of starting dione.

3,3-Ethylenedioxy-16α,17α-epoxy-16β-methylpregn-5-en-20-one (13)

To a soln of the above enone (12; 4.8 g, 0.013 mole) in CH_2Cl_2 (30 ml) and 95% EtOH (150 ml) was added 30% H_2O_2 aq (22.23 ml; 6.61g, 0.19 mole) and 10% NaOH aq (15.4 ml; 1.54 g, 0.0387 mole) at room temp (25-32°), with manual mixing. The clear, homogenous reaction mix was set aside at room temp for 48 hr (by this time $\nu_{C=O}$ had steadily shifted from 1658 cm^{-1} to 1695 cm^{-1}, indicating thereby the disappearance of the starting enone). At the end of this period the reaction mixture was diluted with water (700 ml) and extracted with CH_2Cl_2 (60 ml x 3). The combined organic phase was washed with water (100 ml x 2) and dried. On solvent removal, a solid (5.0 g, m.p. 176-178°) was obtained, which was recrystallised from EtOAc, m.p. 182-184° (4.8 g). IR (KBr): 3400, 1695, 1452, 1442, 1422, 1380,1370, 1360, 1315, 1305, 1260, 1230, 1138, 1098, 1060, 1022, 978, 968, 942, 888,862, 820, 810 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 1.03, 1.03, 1.41 ppm), MeCO (3H,s, 2.19 ppm), OCH_2CH_2O (4H, s, 3.93 ppm), CH=C (1H, m, 5.25-5.40 ppm). (Found: C, 74.53; H, 8.76; $C_{24}H_{34}O_4$ requires: C, 74.57; H, 8.87%).

3,3-Ethylenedioxy-16α-methylpregn-5-ene-17α,20ξ-diol (14)

To a stirred and refluxing soln of LAH (807 mg, 0.021 mole) in THF (80 ml) was added a soln of the above epoxide (4.082 g, 0.011 mole) in THF (40 ml) dropwise (40 min) and the reaction continued for another 7 hr. The reaction mixture was next cooled (0°), moist ether (20 ml), followed by 20% Rochelle salt aq soln (200 ml, cold) added, and the granular ppt filtered, washed with ether (20 ml). Ether phase was separated, the aq part extracted with ether (35 ml x 3), and the combined extracts washed with water and dried. Solvent was flashed off to get a product (4.2 g), which was chromatographed over SiO_2-gel/II (3 cm x 70 cm; 125 g). Elution was carried out with 15% EtOAc in pet. ether(100 ml cuts). First 100 ml eluted (150 mg) a complex mixture which was discarded. Next 2500 ml gave the required product (2.9 g), which was crystallised from MeOH aq, m.p. 157-173°C (mix. of C-20 epimers?) {Last fraction (500 ml) yielded a product (800 mg) consisting of some three compounds, not readily separable and hence was not investigated further}. IR (KBr):3450, 1450, 1380, 1368, 1260,1200, 1130, 1095, 1082, 1012, 995, 950, 890, 878, 860 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 0.84, 1.00 ppm), MeCH- (3H, d, 0.95 ppm, J=7Hz), MeCHOH (3H, d, 1.16 ppm, J = 8Hz), CHOH (1H, m, 3.8-4.1 ppm), OCH_2CH_2O (4H, s, 3.89 ppm), CH=C (1H, m, 5.3 ppm). Mass: m/z 390 (M^+, 30%), 99(100%), 345 (22%), 327(9%), 100(28%), 55(23%), 45(8%), 43(12%). (Found: C, 71.28; H, 9.71; $C_{24}H_{38}O_4H_2O$ requires: C, 70.55; H, 9.87%).

3,3-Ethylenedioxy-17α-hydroxy-16α-methylpregn-5-en-20-one

To a stirred soln of oxalyl chloride (0.49 ml, 0.5796 g, 0.0045 mole) in dry CH_2Cl_2 (8 ml), chilled to -60°, was introduced a soln of DMSO (0.64 ml, 0.703 g, 0.009 mole) in CH_2Cl_2 (2 ml) dropwise over a period of 5 min, while keeping the temp below -58°. The whitish soln was stirred for 40 min at -60°, and at the end of this period a soln of the above diol (1.1126g, 0.0028 mole) in CH_2Cl_2 was added dropwise over a 10 min period, while maintaining the temp. at ~-60°. After stirring at this temp for 1 hr, triethylamine (2 ml) was added and the reaction mixture

stirred for another 1 hr at this temp., after which it was allowed to warm to room temp., diluted with water (20 ml) and the organic phase separated. The aq phase was extracted with CH_2Cl_2 (6 ml x 2), the combined extracts washed with water (15 ml x 4) and dried. Solvent was flashed off to get a product (1.094 g), which was passed through a column of SiO_2-gel/II (1.2 cm x 12 cm; 15 g), using 5% EtOAc in pet. ether as the solvent. After rejecting the first 100 ml of eluate, the required product was collected from the subsequent eluates. This material was crystallised from EtOAc, m.p. 210-213°, yield 0.95g. IR (Nujol): 3485, 1705, 1428, 1350, 1335, 1310, 1265, 1238, 1223, 1208, 1195, 1140, 1128, 1115, 1105, 1088, 1065, 1035, 1005, 970, 955, 922, 878, 862, 820, 796, 740, 696 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 0.80, 1.03 ppm), MeCH (3H, d, 0.91ppm, J = 8Hz), MeCO (3H, s, 2.23 ppm), OCH_2CH_2O (4H, s, 3.92 ppm), CH=C (1H, m, 5.35 ppm). Mass: m/z 388 (M^+, 22%), 99(100%), 345(15%), 327(7%), 100(24%), 43(18%). (Found: C, 74.83; H, 9.38; $C_{24}H_{36}O_4$ requires: C, 74.19; H, 9.34%).

17α-Hydroxy-16α-methylpregn-4-ene-3,20-dione(15)

The above ketone (1.2 g, 0.003 mole) in acetone (30 ml) containing a trace of *p*-TSA was left at room temp. (25-30°) overnight (15 hr). Water (4 ml) was added, acetone flashed off, residue diluted further with water (30 ml) and filtered, dried and crystallised from EtOAc, m.p. 182-184°, yield ~100% (Lit.[6]: m.p. 182-184°). IR (KBr): 3460, 1710, 1665, 1615, 1460, 1440, 1360, 1340, 1285, 1275, 1240, 1220, 1200, 1150, 1130, 1000, 970, 955, 925, 900, 888 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 0.80, 1.17 ppm), MeCH (3H, d, 0.89 ppm, J = 8Hz), MeCO (3H, s, 2.21 ppm), CH=C (1H, s, 5.70 ppm). (Found: C, 77.07; H, 9.53; $C_{22}H_{32}O_3$ requires: C, 76.70; H, 9.36%).

21-Acetoxy-17α-hydroxy-16α-methylpregn-4-ene-3,20-dione (4)

A mixture of the above diketone (15; 641 mg, 0.0018 mole) isopropanol (22 ml) and pyrrolidine (1 ml, 0.021 mole) was refluxed (N_2) and slowly distilled over a period of 1 hr to collect ~5 ml of distillate (comprising of H_2O, isopropanol and pyrrolidine). The residue was cooled to ~40° and the remaining isopropanol and pyrrolidine removed under suction. The yellowish solid residue was collected, washed with pet. ether and dried to get a material (16[28]; 694 mg), m.p. 120-160° (dec.) ^{1}H-NMR: Me (3H singlets at 0.82, 1.0 ppm), MeCH (3H, d, 0.89 ppm, J = 8Hz), MeCO (3H, s, 2.22 ppm), N-CH_2 (4H, m, 3.12 ppm), CH=C (1H, s, 4.78 ppm; 1H, m, 5.0 ppm). This material was used as such in this next step.

To a stirred suspension of the dienamine 16 (620 mg, 0.0015 mole) in EtOH (17 ml) was introduced EtOH-HCl (3 ml, 0.26 g HCl per ml of soln) at room temp. (~26°). A soln of bromine (295 mg, 0.0018 mole) in EtOH (4.5 ml) was made at -60° immediately prior to use. This soln was, next, added dropwise to the above eniminium salt soln at a rate that this bromine colour never persisted for more than a few seconds (~5 min total time). After stirring the reaction mixture for another half an hr, ethanol and gases were removed under reduced press at <40°. The residue was triturated with ether and the pptd solid collected. This solid was redissolved in EtOH, 20% $KHCO_3$ aq (5 ml) added and the mixture stirred for 1 hr. Alcohol was then removed under vacuo and the residue treated with water (70 ml) and the pptd solid (600 mg) collected after several hr. ^{1}H-NMR: $COCH_2Br$ (2H, ABq, 4.2 ppm, J = 14 Hz).

The above crude bromide (574 mg, 0.0013 mole, KOAc (720 mg, 0.0073 mole) and dry acetone were mixed and refluxed (N_2) with stirring for 2 hr. At the end of this period water (4 ml) was added, most of acetone distilled off (reduced press), the residue diluted with H_2O (25 ml) and the mixture left as such overnight. The pptd material (brownish) was collected by filtration, dried and passed through a short bed of SiO_2-gel/II to get a white solid (320 mg), which was crystallized from MeOH, m.p. 158-160° (Lit.[6]: m.p. 158-160.5°). IR (Nujol): 3420, 1750, 1730, 1666, 1611, 1410, 1245, 1138, 1080, 1070, 1025, 920, 870 cm^{-1}. ^{1}H-NMR: Me (3H singlets at 0.79, 1.18 ppm), MeCH (3H, d, 0.92 ppm, J = 8Hz), MeCOO (3H, s, 2.14 ppm), CH_2OAc (2H, ABq, 4.9 ppm, J = 17Hz), CH=C (1H, s, 5.73 ppm).

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L44 ANSWER 96 OF 116 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
AN 1983-03867 BIOTECHDS
TI Chemistry of some ayurvedic crude drugs;
shataavari and **guggulu** with antioxytocin hypocholesterolemic and antiinflammatory activity; isolation and structure determination shataavarin-I **guggulsterol**-II and **guggulsterol**-III
AU Dev S
LO Malti-Chem Research Centre, Nandesari, Vadodara, India.
SO Proc.Conf.Chem.Biotechnol.Biol.Active Nat.Prod.; (1981) 1 Meet., Vol.1, 168-87
DT Journal
LA English
AB Ayurveda, an ancient Indian system of health-management, involves treatments mostly based on plant materials. The evaluation of 2 crude drugs used is presented. The common names of the drugs are, shataavari and **guggulu**. Shataavari comprises the roots of a plant identified as Asparagus racemosus. The ethanol extract of the roots yields a complex mixture which can be extracted further to yield shataavarin I, II, and IV. Only Shataavarin I (13) has antioxytocin activity. **Guggulu** is the resinous exudate from the tree **Commiphora mukul**. The ethyl acetae-soluble portion carries hypocholesterolemic and antiinflammatory activity. The hypocholesterolemic activity is attributable to ketones and that of antiinflammatory is attributable to acids. Important ketones isolated and characterized are: C21 **steroids** - Z-glggulsterol, (20S)-20-hydroxy- 4-pregnene- 3-one, (20R)-20-hydroxy- 4-pregnene-3-one, **guggulsterol**-VI, Z-**guggulsterone** and E-**guggulsterone**; C27 sterols - **guggulsterol** II, and **guggulsterol** III. (35 ref)
TI Chemistry of some ayurvedic crude drugs;
shataavari and **guggulu** with antioxytocin hypocholesterolemic and antiinflammatory activity; isolation and structure determination shataavarin-I **guggulsterol**-II and **guggulsterol**-III
AB. . . plant materials. The evaluation of 2 crude drugs used is presented. The common names of the drugs are, shataavari and **guggulu**. Shataavari comprises the roots of a plant identified as Asparagus racemosus. The ethanol extract of the roots yields a complex. . . mixture which can be extracted further to yield shataavarin I, II, and IV. Only Shataavarin I (13) has antioxytocin activity. **Guggulu** is the resinous exudate from the tree **Commiphora mukul**. The ethyl acetae-soluble portion carries hypocholesterolemic and antiinflammatory activity. The hypocholesterolemic activity is attributable to ketones and that of antiinflammatory is attributable to acids. Important ketones isolated and characterized are: C21 **steroids** - Z-glggulsterol, (20S)-20-hydroxy- 4-pregnene- 3-one, (20R)-20-hydroxy- 4-pregnene-3-one, **guggulsterol**-VI, Z-**guggulsterone** and E-**guggulsterone**; C27 sterols - **guggulsterol** II, and **guggulsterol** III. (35 ref)
CT SHATAAVARIN I, II, VI, **GUGGULSTEROL** I AND III ISOL. STRUCT. DET. CHARACTERIZATION, CRUDE SHATAAVARI AND **GUGGULU** ANTIOXYTOCIN, HYPOCHOLESTEROLEMIC AND ANTIINFLAMMATORY ACT.

L44 ANSWER 88 OF 116 NAPRALERT
AN 92:87715 NAPRALERT
DN T10180
TI CHEMISTRY OF RESINOUS EXUDATES OF SOME INDIAN TREES
AU DEV S
CS MALTI CHEM RES CENT, VADODARA GUJURAT 391340 INDIA
SO PROC INDIAN NATL SCI ACAD (1983) 49 (3) p. 359-385.
DT General review; (Scientific review paper)
LA ENGLISH
CHC 4192
ORGN Class: DICOT Family: BURSERACEAE Genus: COMMIPHORA Species: MUKUL
Synonym(s): BALSAMODENDRON MUKUL
Common name(s): **GUGGULU**; **GUGGUL**
Organism part: GUM RESIN
Geographic area (GT): INDIA; SAS
TYPE OF STUDY (STY): FOLKLORE Classification (CC): ANTIINFLAMMATORY ACTIVITY
Extract. . .
PROPANOIC ACID DOCOSANE-1-2-3-4-TETRAOL-1-YL ESTER,3-(4-HYDROXY-3-METHOXY-PHENYL)
Class identifier (CI): LIGNAN
COMPOUND. Chemical name (CN): CHOLESTEROL
CAS Registry Number (RN): 57-88-5
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTEROL,CIS**
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTERONE,CIS**
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTERONE,TRANS**
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTEROL VI**
CAS Registry Number (RN): 61391-01-3
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTEROL I**
CAS Registry Number (RN): 39025-25-7
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTEROL II**
CAS Registry Number (RN): 39025-26-8
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTEROL III**
CAS Registry Number (RN): 39025-27-9
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): PREGN-4-EN-3-ONE,20(S)-HYDROXY
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): PREGN-4-EN-3-ONE,20(R)-HYDROXY
Class identifier (CI): **STEROID**

L44 ANSWER 116 OF 116 NAPRALERT
AN 92:97346 NAPRALERT
DN W03664
TI CHEMICAL INVESTIGATION OF **COMMIPHORA MUKUL**
AU ALI M A; HASAN M
CS PAKISTAN COUNCIL SCI IND RES, KARACHI PAKISTAN
SO PAK J SCI IND RES (1967) 10 (1) p. 21-23.
DT Journal
LA ENGLISH
OS CA 68:47009
CHC 548
TI CHEMICAL INVESTIGATION OF **COMMIPHORA MUKUL**
ORGN . . .
PAKISTAN; SAS
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): SITOSTEROL,BETA
CAS Registry Number (RN): 83-46-5
Class identifier (CI): **STEROID**

AN 1999:856 NAPRALERT
DN H22818
TI **STEROIDS** AND TERPENOIDS FROM THE GUM RESIN OF AILANTHUS GRANDIS
AU HUNG T; STUPPNER H; ELLMERER-MULLER E P; SCHOLZ D; EIGNER D; MANANDHAR M P
CS INST PHARMAKOG, NIV INNSBRUCK, INNSBRUCK A-6020 AUSTRIA
SO PHYTOCHEMISTRY (1995) 39 (6) p. 1403-1409.
DT (Research paper)
LA ENGLISH
CHC 1540
TI **STEROIDS** AND TERPENOIDS FROM THE GUM RESIN OF AILANTHUS GRANDIS

Microfilm

ORGN . . . . GRANDIS
Organism part: DRIED GUM RESIN
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): PREGNA-4-TRANS-17(20)-DIEN-3-ONE,16-BETA-ACETYL-OXY
Class identifier (CI): **STEROID**
Yield: 00.0158%
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): PREGN-17(20)-CIS-EN-16-ONE,5-ALPHA:3-ALPHA-ACETYL-OXY
Class identifier (CI): **STEROID**
Yield: 00.0035%
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): PREGNAN-16-ONE,5-ALPHA:3-ALPHA-ACETYL-OXY
Class identifier (CI): **STEROID**
Yield: 00.0035%
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): PREGN-4-ENE-3-16-DIONE,20(S)-ACETYL-OXY
Class identifier (CI): **STEROID**
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): **GUGGULSTERONE,CIS**
Class identifier (CI): **STEROID**
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): **GUGGULSTERONE,TRANS**
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): **GUGGULSTEROL I**
Class identifier (CI): **STEROID**
COMPOUND. Chemical name (CN): CHOLEST-4-EN-3-ONE
Class identifier (CI): **STEROID**

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STEROIDS AND TERPENOIDS FROM THE GUM RESIN OF *AILANTHUS GRANDIS*

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Key Word Index—*Ailanthus grandis*; Simaroubaceae; pregnane; gammacerane; structure elucidation.

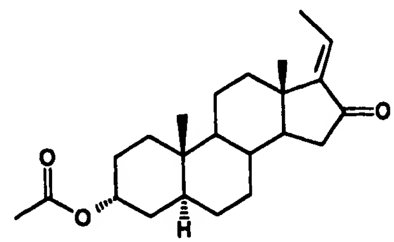
Abstract—From the gum-resin of *Ailanthus grandis* six pregnane, two cholestane, two hopane, one lupane and one gammacerane derivatives have been isolated. One, 3-α-acetyloxy-5-α-pregnan-16-one, is a new compound. Four, 20*S*-acetyloxy-4-pregnene-3,16-dione, 16-β-acetyloxy-pregn-4,17(20)-*trans*-dien-3-one, gammacerane-3,21-dione and 3-α-acetyloxy-5-α-pregn-17(20)-(*cis*)-en-16-one, are new natural products. Their structures were established by UV, IR, MS, ^{1}H and ^{13}C NMR spectroscopy and two-dimensional NMR techniques (COSY, HMQC and HMBC).

INTRODUCTION

Ailanthus grandis Prain is a lofty tree (30–45 m in height) growing in India, Vietnam, Thailand and China [1–3]. In traditional medicine in Nepal and Northern India, the gum-resin from the trunk of this plant (known as 'Gokul-dhup') has been used for the treatment of boils and pimples (M. P. Manandhar, personal communication).

Previous phytochemical investigations of *A. grandis* resulted in the isolation of two quassinoids, 6-α-tigloyl-xy-chaparrinone and 6-α-tigloyloxy-chaparrin, which exhibited significant cell growth inhibition in the P-388 cell line assay [4, 5]. No phytochemical, or pharmacological data have been available so far for the gum resin.

This communication refers to the isolation and structure elucidation of five new natural compounds: 16-β-acetyloxy-pregn-4-17(20)-*trans*-dien-3-one (1), 3-α-acetyloxy-5-α-pregn-17(20)-(*cis*)-en-16-one (2), 3-α-acetyloxy-5-α-pregnan-16-one (3), 20*S*-acetyloxy-4-pregnene-3,16-dione (4), gammacerane-3,21-dione (5). Also studied are *Z*- (6) and *E*-guggulsterone, guggulsterol [6], 22-hydroxy-hopanone-3 [7–9], hop-17(21)-ene-3-one [10], cholest-4-ene-3-one [11, 12], lup-20(29)-ene-3-one-16-ol (resinone) [13], 1,5,9-trimethyl-1,5,9-cyclododecatriene and cembrene [14, 15].



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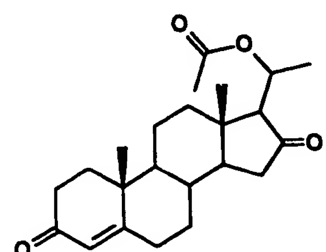

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RESULTS AND DISCUSSION

Fractionation of the gum resin exudate from *A. grandis* by gel filtration (Sephadex LH 20), silica gel and reversed phase chromatography, afforded the compounds 1–14.

Guggulsterone (pregn-4,17(20)-*trans*-diene-3,16-dione) (6), one of the major constituents of the gum-resin, was identified by X-ray analysis. Although this compound has been described in the literature as a constituent of the gum resin of *Commiphora mukul* (Hook. ex Stocks) Engl. (Burseraceae) [6], neither X-ray data nor complete NMR spectral data have been available. A perspective drawing of the compound is given in Fig. 1, the complete NMR assignments are shown in Tables 1 and 2.

The CI and EI mass spectra of 1 displayed $[M + H]^+$ and $[M]^+$ peaks at m/z 357 and 356, respectively, indicating the molecular formula of $C_{23}H_{32}O_3$. The

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^{13}C NMR spectrum sh wed the presence of 23 carbon signals. Six of these carbons were quaternary, six were tertiary, seven were secondary and four were primary. The spectra also exhibited the presence of a carbonyl carbon (δ199.3), two double bonds (-CH=C <) (δ124.0, 170.7 and 118.4, 148.7), a carbinyl carbon (δ72.9) and one carb xyl carbon (δ170.7) assignable to an acetyl group (δ_C21.1; δ_H2.02) [16]. The presence f an acetyl xy group was also supported by the lack of a free OH peak in the range 3600–3000 cm^{-1} in the IR spectrum. The complete structure of 1 was established by means of 2D NMR spectroscopy. The double bond was established in positions C-4 and C-5 by an HMBC experiment, which showed cross-peaks between the sp^2 quaternary carbon C-5 and the C-19 methyl protons, as well as the vinylic proton in C-4 and the quaternary carbon C-10. The ketone carbon belonging to the enone system was located at C-3. The C-4 proton appeared as a small doublet at δ5.73 (J = 1.7 Hz) coupling with the *axial* proton of an allylic methylene group at C-6. The C-2 protons, which were in the vicinity of the ketone function, in the ^{1}H–^{1}H COSY spectrum clearly showed cross-peaks with the protons of C-1. The second double bond

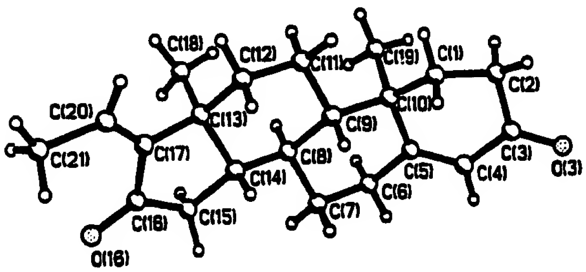

Fig. 1. Perspective view of the structure of compound 6.

Table 1. ^{1}H NMR spectral data of compounds 1–6 ($CHCl_3$-d, δ ppm)*

H	1	2	3	4	5	6
1a	1.7 *m*	1.23 *m*	1.23 *m*	1.72 *m*	1.4 *m*	1.9 *m*
1b	2.00 *m*	1.5 *m*	1.50 *m*	2.00 *m*	1.91 *ddd*	2.05 *m*
2a	2.32 *m*	1.60 *m*	1.64 *m*	2.4 *m*	2.43 *m*	2.4 *m*
2b	s.o.	1.72 *m*	1.71 *m*	2.4 *m*	2.43 *m*	2.5 *m*
3		5.01 *quin*	5.00 *quin*			
4a	5.73 *d* (1.7)	1.5 *m*	1.48 *m*	5.07 *s*		5.70 *m*
4b		1.5 *m*	1.56 *m*			
5		1.5	1.47		1.3 *m*	
6a	2.24 *m*	1.22 *m*	1.20 *m*	2.34 *dd*	1.5 *m*	2.3 *m*
6b	2.24 *m*	s.o.	s.o.	2.4 *m*	1.5 *m*	2.42 *m*
7a	1.01 *m*	1.63 *m*	1.63 *m*	1.1 *m*	1.32 *m*	1.1 *m*
7b	1.83 *m*	s.o.	1.60 *m*	1.8 *m*	1.5 *m*	1.80 *m*
8	1.60 *m*	1.5 *m*	1.52 *m*	1.71 *m*		1.70 *m*
9	0.92 *m*	0.90 *m*	0.92 *m*	1.1 *m*	1.34 *m*	1.1 *m*
11a	1.5 *m*	1.31 *m*	1.32 *m*	1.67 *m*	1.34 *m*	1.50 *m*
11b	1.62 *m*	1.7 *m*	1.63 *m*		1.6 *m*	1.72 *m*
12a	1.20 *m*	1.34 *m*	1.34 *m*	1.4 *m*	1.34 *m*	1.4 *m*
12b	1.83 *m*	1.9 *m*	1.89 *m*	2.00 *m*	1.6 *m*	s.o.
13					1.34 *m*	
14	0.90 *m*	1.4 *m*	1.44 *m*	1.41 *m*		1.4 *m*
15a	1.26 *m*	2.21 *m*	1.73 *m*	2.00 *m*	1.32 *m*	2.20 *m*
15b	2.40 *m*	s.o.	2.20 *m*	2.20 *m*	1.5 *m*	s.o.
16a	5.60 *t* (7.3)				1.5 *m*	
16b					1.5 *m*	
17			1.64 *m*	2.0 *m*	1.3 *m*	
18	0.94 *s*	0.83 *s*	0.67 *s*	0.81 *m*		0.93 *s*
19a	1.2 *s*	1.00 *s*	0.81 *s*	1.2 *s*	1.4 *m*	1.21 *s*
19b					1.91 *ddd*	
20a	5.32 *dq* (1.6 7.6)	6.58 *q* (7.3)	1.22 *m*	5.10 *q* (6.1)	2.43 *m*	5.7 *q* (7.1)
20b			1.63 *m*		2.43 *m*	
21	1.6 *d* (7.6)	1.83 *d*	1.00 *t* (7 3)	1.33 *d*		2.1 *d* (7.1)
23/30					1.1 *s*	
24/29					1.02 *s*	
25/28					0.92 *s*	
26/27					1.0 *s*	
CH_3CO	2.03 *s*	2.03 *s*	2.03 *s*	2.0 *s*		

*Assignments were confirmed by 2D ^{1}H COSY, HMQC and HMBC experiments.
†Coupling constants (J, Hz) are given in parentheses; s.o.: signal totally bscured by other signals.

Table 2. ^{13}C NMR data of compounds 1–6 (in $CHCl_3$-*d*, δ ppm)*

C	1	2	3	4	5	6
1	35.7 *t*	32.9^{a} *t*	32.8^{a} *t*	35.5 *t*	39.5 *t*	35.5 *t*
2	33.9 *t*	26.1 *t*	26.1 *t*	33.8 *t*	34.1 *t*	33.8 *t*
3	199.3 *s*	70.0 *d*	70.0 *d*	199.0 *s*	217.8 *s*	199.1 *s*
4	124.0 *d*	32.6^{a} *t*	32.7^{a} *t*	124.2 *d*	47.3 *s*	124.1 *d*
5	170.7 *s*	40.0 *d*	40.1 *d*	170.4 *s*	54.9 *d*	170.2 *s*
6	32.7 *t*	28.1 *t*	28.1 *t*	32.5 *t*	19.6 *t*	32.5 *t*
7	31.4 *t*	31.9 *t*	32.1 *t*	31.9 *t*	32.5 *t*	31.8 *t*
8	35.0 *d*	34.2 *d*	34.5 *d*	34.2 *d*	41.8 *s*	34.6 *d*
9	54.0 *d*	54.0 *d*	54.3 *d*	53.4 *d*	49.7 *d*	53.6 *d*
10	38.7 *s*	36.0 *s*	36.0 *s*	38.6 *s*	36.8 *s*	38.7 *s*
11	20.7 *t*	20.6 *t*	20.3 *t*	20.2 *t*	21.8 *t*	20.6 *t*
12	35.8 *t*	36.4^{b} *t*	38.3^{b} *t*	38.0 *t*	21.8 *t*	35.4 *t*
13	43.1 *s*	43.4 *s*	42.1 *s*	41.7 *s*	49.7 *d*	43.0 *s*
14	50.8 *d*	50.2 *d*	50.7 *d*	49.9 *d*	41.8 *s*	4.0 *d*
15	33.2 *t*	37.9^{b} *t*	38.5^{b} *t*	38.8 *t*	32.5 *t*	39.2 *t*
16	72.9 *d*	206.4 *s*	219.5 *s*	213.9 *s*	19.8 *t*	207.2 *s*
17	148.7 *s*	148.1 *s*	63.4 *d*	65.9 *d*	54.9 *d*	147.8 *s*
18	19.0 *q*	17.7 *q*	13.4^{c} *q*	13.6 *q*	36.8 *s*	19.5 *q*
19	17.4 *q*	11.1 *q*	11.4 *q t*	17.3 *q*	39.5 *t*	17.3 *q*
20	118.4 *d*	128.9 *d*	17.6 *t*	67.0 *d*	34.1 *t*	130.4 *d*
21	13.4 *q*	13.1 *q*	13.5^{c} *q*	19.9 *q*	217.8 *s*	14.0 *q*
22					47.3 *s*	
23					21.1 *q*	
24					26.6 *q*	
25					15.9 *q*	
26					16.2 *q*	
27					16.2 *q*	
28					15.9 *q*	
29					26.6 *q*	
30					21.1 *q*	
MeCO	170.7 *s*	170.6 *s*	170.6 *s*	169.8 *s*		
MeCO	21.1	21.5 *q*	21.5 *q*	21.3 *q*		

*Assignments were confirmed by DEPT and 2D ^{1}H-^{13}C one-bond as well as long-range correlation experiments; assigments may be interchangeable within vertical column.

was located between C-17 and C-20. The acetyloxy group was positioned at C-16 by its ^{1}H–^{1}H and ^{1}H–^{13}C interactions. The configuration was established as *trans* (*Z*) by comparing the chemical shift values for the C-18 methyl group of 1 with the corresponding NMR data of *Z*- (6) and *E*-guggulsterone. According to Benn and Dodson [16], the orientation of the acetyloxy group at C-16 in the *Z*-isomer can be established by the ^{1}H NMR shift value of the C-18 methyl group. In comparison with the 16-oxo-derivative, an α-oriented acetyloxy group at C-16 causes a larger downfield shift (0.18 ppm) than a β-oriented one (nearly unaffected < 0.06 ppm). The shift difference between H-18 of 1 and H-18 of *Z*-guggulsterone (6) was 0.009 ppm. Consequently, the orientation of the acetyloxy group at C-16 was determined as β. The structure of 1 was thus identified as 16-β-acetyloxy-pregn-4,17(20)-*trans*-dien-3-one. The unambiguous assignments f r carbons and protons of 1 are sh wn in Tables 1 and 2, ^{1}H–^{13}C long-range connectivities in Fig. 2. Compound 1 has been kn wn only as a partially synthetic compound [16]; NMR data have n t been reported.

The CI mass spectrum of the acetyloxy-pregnen-one derivative (2) showed a $[M+H]^+$ peak at *m/z* 359, indicating the molecular formula $C_{23}H_{34}O_3$. Other prominent fragments appeared at *m/z* 299 $[M+H-HOAc]^+$, 203, 177, 149, 107 and 73. ^{1}H and ^{13}C NMR data (Tables 1 and 2) exhibited the presence of a >C=CH– group (δ_C148.1, 128.9; δ_H6.5, 1H, *q*, $J = 7.3$ Hz), a ketone function in a 5-membered ring (δ_C206.4) an acetyl group (δ_C170.6 and 21.5; δ_H2.03 (3H, *s*)) and one carbinyl carbon (δ_C70.0). Placement of the ketone function in position C-16 and the double bond between C-17 and C-20 was established by comparison of the NMR data with those of *E*- and *Z*-guggulsterone. The appearance of a vinyl proton (H_{20}) as a quartet at δ6.48 ($J = 7.3$ Hz) and a doublet of three methyl protons (C-21 methyl group) at δ1.83 ($J = 7.3$ Hz) in the ^{1}H NMR spectrum confirmed this assignment. The *cis* (*E*)-configuration of the C-17 (20) double bond was established by comparison of the ^{13}C NMR shift value of the C-18 methyl carbon signal with that of *E*-guggulsterone. ^{1}H and ^{13}C NMR data for ring C, D and side chain carbons were in good agreement with those of

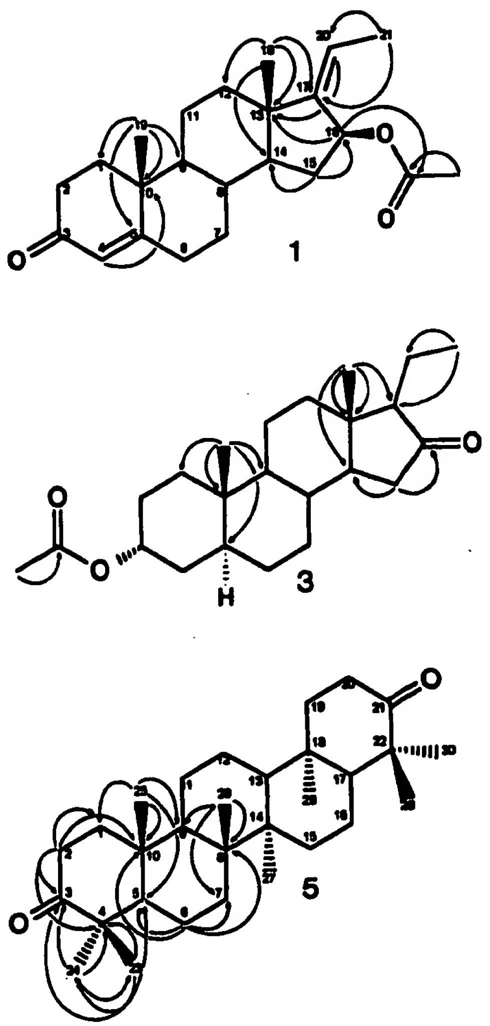

Fig. 2. Most significant correlations observed in HMBC spectra of compounds **1**, **3** and **5**.

E-guggulsterone. The stereochemistry of the ring A/B junction was deduced from the carbon chemical shift of the C-19 methyl group. It is well known that the C-19 methyl signal of A/B *trans*-steroids is upfield shifted by 11–12 ppm compared with that of their 5β-counterparts, the C-19 resonance of which appears around δ22–24 [17, 18]. The C-19 methyl carbon signal appeared at δ11.1, indicating that **2** belonged to the 5α-pregnane series. The acetyloxy group was located at C-3 of the ring A by comparison with 5α-pregnane, taking into account the α and β effects of the OAc group to vicinal carbons. Accordingly, the carbon signals of C-1, C-2, C-4, and C-5 of **2** are shifted by -6.1, 3.7, 3.4 and -7.2 ppm, respectively, relative to those of 5α-pregnane [11]. The configuration of the acetyloxy group was established by the ^{1}H NMR spectrum with the half-height band width ($W_{1/2}$) of 7.1 Hz. This coupling pattern requires the interactions of an equat rial proton (β-oriented in this case) with four vicinal prot ns [19, 20]. The acetyloxy group was therefore established as an α-oriented one. Assignments f r the carbons in rings A and B were in excellent agreement with those for equivalent structures in 5α-steroid series [11]. Therefore, compound **2** is 3α-acetyloxy-5α-preg-17(20)-*cis*-en-16-one. There is nly one report dealing with partial synthesis of this compound [21]. This is the first time this compound has been found as a constituent of a plant.

The CI mass spectrum of **3** showed a $[M + H]^+$ peak at *m/z* 361, indicating the molecular formula of $C_{23}H_{36}O_3$. Other prominent fragments appeared at *m/z* 343 and 301 $[M + H - HOAc]^+$. The ^{13}C NMR spectrum of **3** in comparison with that of **2** showed the lack of a double bond in the side chain. This was supported by the appearance of three methyl protons as a triplet ($\delta_H = 1.00$, $J = 7.3$ Hz) and the absence of the vinyl proton signal in the ^{1}H NMR spectrum. Of the functional carbons, only one ketone function (δ_C219.5), one carbinyl carbon (δ_C70.0) and one acetyl group (δ_C170.6, 21.5; δ_H2.03 (3H, *s*) were observed. The location of the acetyloxy group was established at C-3 by comparing the carbon resonances of the rings A and B with those of **2** (Table 2). The A/B ring junction was determined as *trans*- (i.e. 5α-pregnane derivative) by the shift value of the C-19 methyl carbon. The acetyloxy group in position C-3 was established as an α-oriented substitute from the fact that the pseudoquintet of its geminal proton again showed a relative small half-height band width ($W_{1/2} = 7.5$ Hz). Assignments for carbon shift values of **3** were achieved by the aid of 2D NMR spectroscopy. The ketone function was localized at C-16. In the HMBC spectrum, two protons of the C-15 methylene carbon (δ_H1.73, 2.20) showed cross-peaks with signals of the carbonyl carbon (δ_C219.5) and the signals of the methine carbon assigned to C-14. The carbon and proton shift values are shown in Tables 1 and 2. Thus, compound **3** is 3α-acetyloxy-5α-pregnan-16-one. The 3β-acetyloxy isomer has been obtained by chemical synthesis [21]; the 3α-acetyloxy isomer to our knowledge is a new compound.

The molecular formula of **4** was determined as $C_{23}H_{32}O_4$ based on CIMS (*m/z* 373 $[M + H]^+$). Inspection of the ^{1}H and ^{13}C NMR data showed that **4** has the same pregnene skeleton as *Z*-guggulsterone (**6**) with carbonyl functions in positions C-3 and C-16, methyl groups at C-10, C-13 and C-20 and a 4,5 double bond. In comparison to guggulsterone, compound **4** has an additional acetyloxy group at C-20 and no 17, 20 double bond. The position of the acetyloxy group was located by an ^{1}H–^{1}H COSY experiment. The C-21 methyl prot ns showed a coupling, with one proton of the methine carbon bearing an oxygen atom (δ_C67.0). This proton (δ_H5.10, $J = 6.4$ Hz) in turn coupled with the methine proton assigned to C-17 (δ_H2.00; δ_C65.9). The complete assignments of carbon and proton shift values of **4** are shown in Tables 1 and 2. The 20*S*-stereochemistry of **4** was established by comparing the ^{13}C NMR shift values of C-20 (δ67.0), C-21 (δ19.9) and C-17 (δ65.9) with the corresponding signals f 3β,20*S*-diacetyloxy-5-α-pregnan-16-one [22]. Thus, the structure f **4** is 20*S*-acetyloxy-4-pregnene-3,16-dione. Alth ugh **4** has been partially synthesized from 20-hydroxy-4-pregnene-3,16-dione [23], this is the first report of the isolati n of this

compound from natural sources. It is also the first report of its ^{13}C NMR data.

The FAB mass spectrum of compound 5 (m/z 447 $[M + Li]^+$) suggested the m lecular formula of $C_{30}H_{48}O_2$ and the presence of a triterpenic skeleton. Although the molecular formula was attributable to a triterpene, the ^{13}C and ^{1}H NMR spectra of 5 revealed only 15 carbon and 24 hydrogen signals involving four angular methyl groups, five methylene, two tertiary and four quaternary carbons. The existence of a signal at δ217.8 in the ^{13}C NMR spectrum indicated that one quaternary carbon belonged to a ketone function group. The lack of signals in the range of δ100–150 in the ^{13}C NMR spectrum and in the range higher than δ2.6 in the ^{1}H NMR spectrum indicated the absence of carbon–carbon double bonds. The results from the NMR spectra corresponded to a formula of $C_{15}H_{24}O$, a half molecule of 5. This indicated that 5 has a symmetric structure [24, 25]. The complete structure of the 'half molecule' of 5 was obtained from 2D NMR experiments. The HMBC spectrum showed cross-peaks between ^{1}H and ^{13}C NMR signals of two methyl groups (δ_H1.02, δ_C26.6 and δ_H1.06, δ_C21.1), which could be assigned to C-24 and C-23, respectively [26, 27]. In addition, the two methyl group signals showed cross-peaks with the carbon resonances of a quaternary carbon (δ_C47.3), a keto-carbon (δ_C217.8) and a methine carbon (δ_C54.9). Thus, the methyl groups had to be located at the C-4 position and the ketone group and the methine carbon could be established as C-3 and C-5, respectively, which are usual f r triterpenic compounds. The proton signal of another methyl group (δ_H0.92, δ_C15.9), which showed a cross-peak with the C-5 methine carbon resonance, was assignable to C-25. This was confirmed by additional cross-peaks with another tertiary (δ_C49.7), a quaternary (δ_C36.8) and a methylene carbon (δ_C39.5) attributable to C-9, C-10 and C-1, respectively. Similarly, the position of the C-26 methyl group was established. Protons of this group exhibited interactions with the carbons C-9, C-7 and C-8. Signal assignments to C-1, C-2 and C-6 were also achieved by 2D NMR spectra. In the ^{1}H–^{1}H COSY spectrum the two protons of C-2 in the vicinity of the ketone group (δ_H2.43, 2H, *m*) showed couplings with the tw protons of C-1 (δ_H1.91 and 1.39). In addition, in the HMBC spectrum, the ^{1}H NMR signals of C-2 revealed a cross-peak with the carbon resonances of C-3, C-1, C-4 and C-10. A carbon that resonated at δ19.8 (δ_H1.46) was assigned to C-6 because its proton signal showed cross-peaks with the ^{13}C NMR resonances of C-5, C-7, C-8 and C-10. The remaining methylene carbon signal (δ_C21.8, δ_H1.34, 1.55, *m*) could be assigned to C-11. Thus, compound 5 is gammaceran-3,21-dione. Assignments of all ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2. A literature survey indicated that only two reports [28, 29] have dealt with the partial synthesis of gammaceran-3,21-di ne by xidati n f a corresponding di l. To the best of ur kn wledge, this is the first report of the isolation of 5 from natural s urces. This is also the first time the ^{1}H and ^{13}C NMR shift values of 5 are documented.

The occurrence of *Z*-(6) and *E*-guggulsterone (7) together with guggulsterol-I in the gum resins of *A. grandis* and *C. mukul* may show a certain chemical relationship between the Simaroubaceae and the Burseraceae families. From the botanical point of view, they are closely related families. However, both resins can easily be distinguished by their terpenic constituents, such as hopane, lupane and gammacerane derivatives, which are present in the resin of *A. grandis* but absent in that of *C. mukkul*. The presence of cholestane derivatives, especially 20,22-dihydroxy-cholestan-4-ene-3-one, in the resin of *A. grandis* supports the biosynthetic pathway of C_{21}-steroids in plants from cholestane derivatives via a 20,22-dihydroxy intermediate [6, 30–33]. In nature, gammacerane derivatives (e.g. compound 5) have been found in crude petroleum, petroleum source rocks and geological sediments. In the plant kingdom they are found only rarely [24].

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra (δ, ppm, J in Hz) were recorded with a Bruker AM 300 (300/75 MHz) using $CHCl_3$-*d* as solvent and internal standard. EIMS spectra were recorded on a Mat 44/S (Finigan) or a Kratos MS 80 RFA mass spectrometer. Chemical ionization (CI) mass spectra were run on the Mat 4415. Fast atom bombardment mass spectra (FABMS) were performed on a Kratos MS 80 RFA mass spectrometer. Single crystal X-ray diffraction experiment was performed on a Siemens R3 m/V diffractometer using CuKα radiation (l = 1.54178 Å) with a balance filter monochromator. Data collection was done at 296 K from an orthorhombic crystal using ω scan technique with 2ϕ range of 0.0°–114.0°. Structure was solved by direct method using a Siemens Shelxtl plus (PC version) program system. Melting points are uncorrected. UV spectra were recorded either in MeOH on a Shimadzu UV-160A or on-line by photo diode array detection (Diode array detector L-4500, Merck-Hitachi) in $MeCN/H_2O$ mixtures. Fourier transform infrared spectroscopy (FT-IR) was performed on a Bruker IFS 25 spectrometer connected to a Bruker infrared microscope.

Column chromatography used silica gel (230–400 mesh) (Merck), Sephadex LH 20 (Pharmacia) (MeOH). TLC: silica gel 60 F_{254}, 0.25 mm (Merck), solvent mixtures: *n*-hexane, *n*-hexane–EtOAc (2:1, 4:1), $CHCl_3$–MeOH (9:1). Spray reagents: vanillin (1%) and H_2SO_4 (10%) in EtOH, followed by heating. Dragendorff's reagent. PLC (preparative-layer chromatography): silica gel 60 F_{254}, 1 mm (Merck), solvent mixture: CH_2Cl_2–EtOAc (19:1). Analytical HPLC was carried out with a Supersphere® 100 RP-18 Lichrocart® 250-4, particle size 5 μm, column, Merck; gradient system: 5 min at 48% MeCN, 48–53% MeCN in 15 min, 53–58% MeCN in 10 min, 58–100% MeCN in 30 min; UV detecti n was perf rmed at 210 nm. MPLC used Lichroprep RP-18 silica gel (particle size 40–63 μm, Merck N°13900) as stationary and a mixture f H_2O–MeCN (4:21) as m bile phase.

Plant material. The gum-resin exudate of *A. grandis* was collected in northern India. It was supplied and botanically identified by Dr Manandhar (Department of Forestry and Plant Research, National Herbarium and Plant Laborat ries, Godawary, Lalitpur, Nepal). A herbarium specimen is deposited at the herbarium f the Institut für Pharmakognosie, Universität Innsbruck (A).

Isolation of compounds. Ground resin (100 g) was exhaustively extracted with MeOH (5 × 250 ml) in an ultrasonic bath at room temp. for 15 min. The clear solns after filtering were combined. MeOH was removed *in vacuo* to give 41.5 g of a brown, half-solid residue (41 g), which was redissolved in 250 ml of a mixture of MeOH–H_2O (95:5) and then distributed (12×) with 100 ml *n*-hexane presaturated with MeOH–H_2O. The hexane partitions were pooled together and the combined soln was washed with 25 × 2 ml MeOH–H_2O and dried over anhydrous Na_2SO_4. The solvent was evaporated under red. pres. to give 23.8 g of residue as a yellow thick oil. A sample (23.5 g) was dissolved in a sufficient amount of MeOH to obtain a thick soln. This soln was subjected to Sephadex LH-20 CC (1 ml for each separation). Frs of 10 ml were collected and monitored by TLC. Frs having the same TLC pattern were pooled. MeOH was removed *in vacuo* to give seven frs, A1 to A7. Among these, frs A4 (13.5 g) and A3 (7.5 g) were the two largest. Fr A4 (13 g) was chromatographed on silica gel. Mixtures of hexane and EtOAc (95:5, 9:1, 4:1, 7:3, 3:2 and 1:1), $CHCl_3$ and then $CHCl_3$–MeOH (8:2) were used for elution. Frs having the same TLC patterns were combined and the solvent removed under red. pres. In all, 22 subfrs were obtained as gummy or thick oil residues, four of them yielding after crystallization (EtOAc) pure compounds **6** and **5**. Silica gel CC of subfr. 19 (300 mg) using CH_2Cl_2 and increasing amounts of EtOAc (3, 5 and 10%) and CH_2Cl_2–EtOH; (9:1) as solvent mixtures gave compound **4** which was purified by repeated silica gel CC using $CHCl_3$ and $CHCl_3$–MeOH; (99:1, 49:1), $CHCl_3$–EtOAc; (99:1, 95:5) and *n*-hexane–EtOAc; (19:1 to 4:1) (yield of **6**). Pure compounds **3** (25 mg) and **2** (3.5 mg) were obtained from subfr. 7 (490 mg) by repeated silica gel CC using CH_2Cl_2 with increasing amounts of EtOAc (from 0 to 2%). Subfr. 13 (1.3 g) was subjected to repeated silica gel CC. Elution was carried out with $CHCl_3$–EtOAc; (24:1) and CH_2Cl_2–C_6H_6; (9:1) with increasing amounts of EtOAc (1% up to 15%). Compound **1**, obtained as a mixture with two other compounds (220 mg) was repeatedly chromatographed on reversed-phase MPLC using an azeotropic mixture of H_2O–MeCN as eluent to give the pure substance (15.8 mg).

Compound **1**. Needles; mp 158–160°; λ_{max} nm: 242 (MeCN–H_2O); IR ν_{max} cm^{-1}: 2963, 2937, 2914, 2855, 2836, 1731, 1672, 1613, 1449, 1438, 1373, 1250, 1235, 1190, 1036, 954, 878. CIMS *m/z* (rel. int.): 357 $[M + 1]^+$ (100), 297 $[M + H - HOAc]^+$ (59.5), 147 (5.8), 113 (4.9), 107 (7.9), 73 (25.6); EIMS *m/z* (rel. int.): 356 $[M]^+$ (1.2), 314 (18.9), 299 (30.9), 281 (14.8), 230 (5.9), 173 (12.2), 91 (27.1), 43 (100); ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **2**. Slightly yellow crystals; mp 102–103°; UV λ_{max} nm: 243 (MeCN–H_2O); IR ν_{max} cm^{-1}: 2929, 2882, 2856, 1730, 1649, 1590, 1445, 1371, 1259, 1239, 1193, 1150, 1028, 1013, 980, 924, 852. CIMS *m/z* (rel. int.): 359 $[M + H]^+$ (100), 299 $[M + H - HOAc]^+$ (84.1), 203 (1.3), 177 (1.2), 149 (2.0), 107 (1.4), 73 (9.5): ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **3**. Needles; mp 102–103°; UV λ_{max} nm: 243 (MeCN–H_2O); IR ν_{max} cm^{-1}: 2973, 2938, 2879, 2847, 2865, 1738, 1727, 1650, 1447, 1385, 1364, 1266, 1250, 1166, 1201, 978. CIMS *m/z* (rel. int.): 361 $[M + H]^+$ (46.6), 343 (4.1), 301 $[M + H - HOAc]^+$ (100), 283 (6.1), 203 (2.1), 137 (1.1), 73 (11.4); ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **4**. Cubic crystals; mp 191–193°; UV λ_{max} nm: 241 (MeOH); IR ν_{max} cm^{-1}: 2979, 2943, 2924, 2857, 1733, 1673, 1610, 1457, 1436, 1419, 1367, 1257, 1183, 1101, 1047, 957; CIMS *m/z* (rel. int.): 373 $[M + H]^+$ (6.4), 313 $[M + H - HOAc]^+$ (100), 312 (23.8), 61 (1.0); ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **5**. Crystals; mp 317–318°; IR ν_{max} cm^{-1}: 3011, 2992, 2968, 2947, 2863, 1707, 1482, 1456, 1423, 1387, 1377, 1309, 1225, 1142, 1114, 1070, 1005; FABMS *m/z*: 447 $[M + Li]^+$; ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **6**. Large prisms; mp 189–190°; UV λ_{max} nm: 241 nm (MeOH); IR ν_{max} cm^{-1}: 2963, 2937, 2914, 2855, 1732, 1627, 1313, 1449, 1438, 1373, 1250, 1235, 1036; CIMS *m/z* (rel. int.): 313 $[M + H]^+$ (100), 297 (1.9), 270 (1.2); ^1H and ^{13}C NMR data see Tables 1 and 2.

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CN   Pregna-4,17(20)-diene-3,16-dione (7CI, 9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   Guggulsterone
CN   Gugulipid
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MF   C21 H28 O2
LC   STN Files:   AGRICOLA, ANABSTR, BEILSTEIN*, BIOBUSINESS, BIOSIS,
       BIOTECHNO, CA, CAOLD, CAPLUS, CHEMCATS, CIN, EMBASE, IPA, MEDLINE, PHAR,
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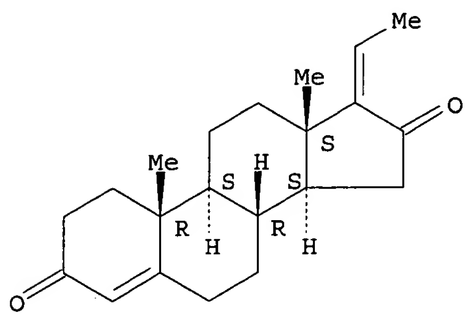

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L22          0 GUGGULSTERONE Z/CN

=> s guggulsterone e/cn
L23          1 GUGGULSTERONE E/CN

=> d

L23  ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2002 ACS
RN   39025-24-6  REGISTRY
CN   Pregna-4,17(20)-diene-3,16-dione, (17E)- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   (-)-(E)-Guggulsterone
CN   E-Guggulsterone
CN   Guggulsterone E
FS   STEREOSEARCH
DR   137682-27-0
MF   C21 H28 O2
LC   STN Files:   AGRICOLA, ANABSTR, BEILSTEIN*, BIOTECHNO, CA, CAPLUS,
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Absolute stereochemistry.
Double bond geometry as shown.
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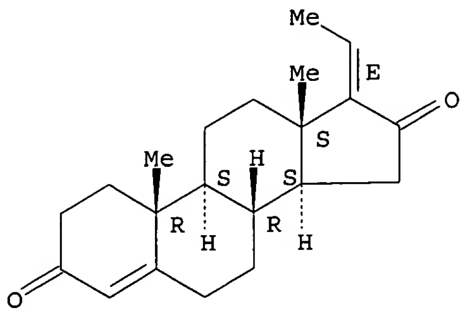

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18 REFERENCES IN FILE CAPLUS (1967 TO DATE)

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DUPLICATE 19
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DN A07160
TI CHEMISTRY OF AYURVEDIC CRUDE DRUGS-I. **GUGGULU** (RESIN FROM **COMMIPHORA MUKUL**)-1: STEROIDAL CONSTITUENTS
AU PATIL V D; NAYAK U R; DEV S
CS NATL CHEM LAB, POONA 8 INDIA
SO TETRAHEDRON (1972) 28 p. 2341.
CHC 1680
TI CHEMISTRY OF AYURVEDIC CRUDE DRUGS-I. **GUGGULU** (RESIN FROM **COMMIPHORA MUKUL**)-1: STEROIDAL CONSTITUENTS
ORGN Class: DICOT Family: BURSERACEAE Genus: COMMIPHORA Species: MUKUL
Synonym(s): BALSAMODENDRON MUKUL
Common name(s): **GUGGULU**
Organism part: FLORETS(DISC)
Geographic area (GT): INDIA; SAS
TYPE OF STUDY (STY): FOLKLORE Classification (CC): ANTIINFLAMMATORY ACTIVITY
Extract type:. . .
Yield: 00.006%
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): CHOLESTEROL
CAS Registry Number (RN): 57-88-5
Class identifier (CI): **STEROID**
Yield: 00.01%
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COMPOUND. Chemical name (CN): **GUGGULSTERONE,CIS**
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Yield: 00.003%
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COMPOUND. Chemical name (CN): **GUGGULSTERONE,TRANS**
Class identifier (CI): **STEROID**
Yield: 00.0025%
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CAS Registry Number (RN): 39025-26-8
Class identifier (CI): **STEROID**
Yield: 00.00018%
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Class identifier (CI): **STEROID**
Yield: 00.00023%
TYPE OF STUDY (STY): ISOLATION
COMPOUND. Chemical name (CN): SI
Class identifier (CI): INORGANIC
COMPOUND. Chemical name. . .

Tetrahedron. Vol. 28, pp. 2341 to 2352. Pergamon Press 1972. Printed in Great Britain

CHEMISTRY OF *AYURVEDIC* CRUDE DRUGS*—I

GUGGULU (RESIN FROM *COMMIPHORA MUKUL*)—1: STEROIDAL CONSTITUENTS†‡

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National Chemical Laboratory, Poona 8, India

(*Received in the UK* 14 *December* 1971; *Accepted for publication* 5 *January* 1972)

Abstract—Guggulu, the gum-resin exudate from the tree *Commiphora mukul* is a complex mixture of steroids, diterpenoids, aliphatic esters, carbohydrates and a variety of inorganic ions, besides minor amounts of sesamin and other unidentified constituents. The present communication discusses in some detail the steroidal constituents, which include, cholesterol, 4,17(20)-(*trans*)-pregnadiene-3,16-dione (I), 4,17(20)-(*cis*)-pregnadiene-3,16-dione (II) and three new sterols—guggulsterol-I, guggulsterol-II and guggulsterol-III which are shown to be VIII, XV and XVI respectively.

GUGGULU (SANSKRIT) IS THE gum-resin exudate from the tree *Commiphora mukul* (Hook, ex Stocks) Engl. (Syn. *Balsamodendron mukul* Hook, ex Stocks) and is an article of commerce in India.[1] The classical Ayurvedic literature claims *guggulu* to be efficacious in the treatment of rheumatoid arthritis, obesity and allied disorders, besides indicating for it several other therapeutic uses.[2] Recent pharmacological studies on the crude drug as well as (in some cases) on some of its fractions and pure constituents, have revealed significant anti-inflammatory, anti-rheumatic[3,4] and hypocholestremic/hypolipaemic[5–8] activity, thus providing at least some support to the ancient claims.

The gum-resin is known to furnish an essential oil ($\sim$0·4%) consisting chiefly of myrcene and "dimyrcene" (camphorene).[9] It has also been separated by alcohol extraction into a soluble resin ($\sim$50%) and an insoluble carbohydrate gum;[10] detailed structural investigations on the carbohydrate gum have been reported.[10–11] It has also been noted[12] that the resin from *Commiphora mukul* is completely devoid of triterpenoids in contrast to the resin from related species *Commiphora glandulosa* Schinz which contains a number of triterpene acids.[13]

For the present investigation, the *guggulu* gum-resin was fractionated by successive solvent triturations into a pet. ether fraction (9–11%), an EtOAc fraction (32–35%) and an EtOAc insoluble residue (54–59%). The EtOAc insoluble residue was found to be free from any glycosides, and is essentially a carbohydrate polymer with a high

* Ayurveda is the ancient Indian system of treating body disorders and infections and is still freely practised in India. Though, almost always, a number of crude drugs go into formulating a specific remedy, quite often one crude drug forms the basis. Many of these drugs have been the subject of scientific investigations, but usually in a rather disjointed manner. In the present series, it is proposed to discuss the chemistry of some of those single drugs which have received support from recent pharmacological/clinical investigations.

† Presented at, *Seminar on Disorders of Lipid Metabolism*, held in New Delhi on October (1971).

‡ Communication No. 1607, National Chemical Laboratory, Poona 8, India.

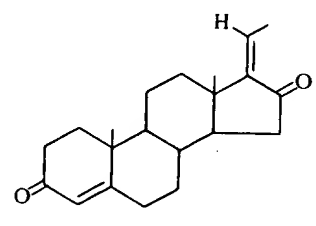
Some local names: Hindi, *guggul*; Marathi, *guggule*.

($\sim$15%) ash content and, in view of the earlier work of Bose and Gupta[10,11] and its toxic character,[14] was not investigated further.

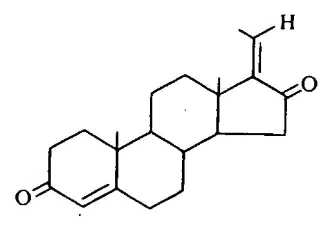
Petrol ether fraction

Systematic chromatography of pet. ether soluble fraction gave, besides some intractable mixtures, a diterpene hydrocarbon ($C_{20}H_{32}$, liq.; 8%*), a diterpene alcohol ($C_{20}H_{34}O$, m.p. 37–38°; 27%), (+)-sesamin[15] (1·2%), cholesterol† ($\sim$2%) and two other isomeric $C_{21}H_{28}O_2$ steroids of m.p. 192–193° (5%) and 168–170° (1·2%). The two diterpenes appear to be new and their structure elucidation will be reported in a subsequent communication.

The $C_{21}H_{28}O_2$ (M^+, *m/e* 312) steroid of m.p. 192–193°, from its spectral characteristics (UV, IR, PMR) has been formulated as 4,17(20)-(*trans*)-pregna-3,16-dione (I), a compound which has recently been synthetically prepared.[18] Though a direct comparison has not been possible, comparison of the physical characteristics (Experimental) and spectral data with that reported in the literature[18] leaves no doubt as to their identity. This was further confirmed by its conversion (Li/liq. NH_3 and then Sarett oxidation) to the known[19] 5α-pregnan-3,16-dione.



I



II

The isomeric $C_{21}H_{28}O_2$ (M^+, *m/e* 312) steroid of m.p. 168–170° was likewise shown to be the *cis*-isomer (II).[18]

Though the natural occurrence of C_{21} steroids is well-known,[20] compounds I and II are being reported occuring in nature for the first time and in accordance with the usual practice are being assigned trivial names, *Z*- and *E-guggulsterone* respectively.*

Ethyl acetate fraction

This material has a high ester number and is very complex in nature and failed to give any useful results on chromatography (SiO_2 gel). Hence, it was saponified and the neutral product ($\sim$55%) systematically chromatographed to furnish, besides additional quantities of *Z*-guggulsterone (0·6%†) and *E*-guggulsterone (0·5%), three new sterols ($\sim$1·2%) and long-chain aliphatic triols (15–20%). The chemistry of the

* Yields computed from chromatography data and are very approximate; the percentage is based on the pet. ether fraction on w/w basis.

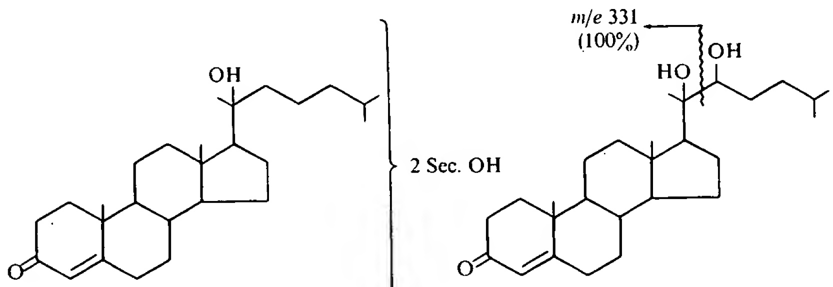
† The occurrence of cholesterol in plant tissues is quite rare and has been noted relatively recently.[16] *Commiphora abyssinica* is the only *Commiphora* species known so far to contain cholesterol.[17]

* The prefixes *Z* and *E* refer to the stereochemistry of the 17(20)-olefinic linkage, according to a recent general proposal.[21]

† These yields are per cent of the total EtOAc fraction (w/w basis).

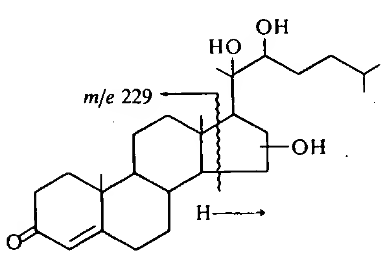
aliphatic triols as well as the nature of the acids (obtained on saponification of the EtOAc fraction) will be discussed in a later publication. The three new sterols have been designated *guggulsterol*-I, *guggulsterol*-II and *guggulsterol*-III and their structure elucidation will now be discussed.

Guggulsterol-I. This compound (m.p. 225–228°) analyses for $C_{27}H_{44}O_4$ (M^+, *m/e* 432) and shows the following structural features: three $\underline{Me}$—$\overset{|}{\underset{|}{C}}$— (PMR in $CDCl_3$: 9H, s, 1·21 ppm), $\underline{Me}_2CH$— (PMR: 6H, *d*, 0·92 ppm, $J = 6$ Hz), two $C\underline{H}OH$ (PMR: two 1H, ill-defined multiplets centred at 3·86 and 4·45 ppm. IR (Nujol): 3300, 1080 cm^{-1}) and —CO—CH=$\underset{|}{C}$— (λ_{max}^{EtOH} 241 nm; ε, 16,650. IR: 1620, 1680 cm^{-1}. PMR: 1H, s, 5·75 ppm). From the mol. formula and functionality revealed above, it is obvious that guggulsterol-I should be tetracyclic and from the nature of the Me signals a steroid nucleus appeared most likely, in which case the fourth oxygen function must be a tertiary OH. From these considerations, part-structure III appeared quite reasonable, the tertiary OH being placed at C_{20} in view of the presence of three $\underline{Me}$—$\overset{|}{\underset{|}{C}}$— groups in guggulsterol-I.

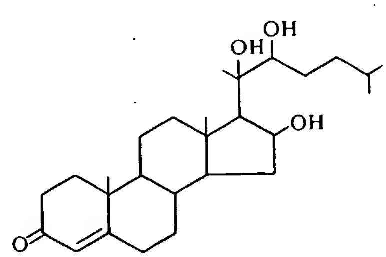


III IV

1 Sec. OH



V

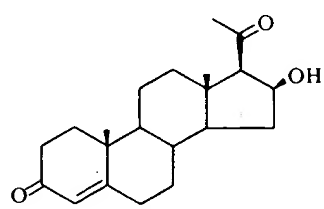


VI

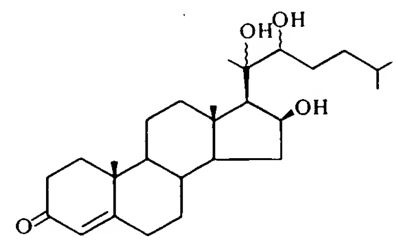
The electron impact induced fragmentation of guggulsterol-I, not only supports formulation III, but also helps in the location of the two secondary OH's. The spectrum shows the base peak at *m/e* 331, arising from cleavage of the side-chain as shown in IV and, this requires that one of the secondary OH's must be placed at C-22

and the second sec OH must be located on one of the rings. That the preferred mode of fragmentation of an α-glycol should involve the bond joining the two OH's, is theoretically expected and has experimental validity.[22] The next major ion, *m/e* 313 (66%) apparently arises from a similar cleavage of (M-18) ion (414–101). The occurrence of an ion at *m/e* 229 (9%) suggests that the ring sec OH should be at C-15/C-16, as the *m/e* 229 ion can arise by the well-established and fairly general steroid fission[23] depicted in V. These considerations lead us to two possible gross structures (VI and the alternative with ring OH at C-15) for guggulsterol-I. Of these VI is preferred because of the pattern of oxidation found in *Z*- and *E*-guggulsterone, which co-occur in the gum-resin. Decisive evidence in support of structure VI, which also helps in the elucidation of the stereochemistry at C-16 and C-17, was obtained as follows.

Guggulsterol-I on interaction with $NaIO_4$ yielded essentially two products, which after chromatographic separation were identified as iso-caproic aldehyde (by comparison of the m.p., IR and PMR spectra of its 2,4-dinitrophenylhydrazone with those of an authentic sample) and 16β-hydroxyprogesterone (VII). Though an



VII



VIII

authentic sample of VII could not be obtained, identity of the cleavage product from guggulsterol-I with 16β-hydroxyprogesterone (VII) was established by comparison of its m.p., $[\alpha]_D$, UV, IR and mass spectra with those reported in the literature.[24] This degradation clearly defines guggulsterol-I as VIII, in which the C-20, C-22 stereochemistry is yet to be elucidated.

Guggulsterol-II. This compound (m.p. 231–233°) analyses for $C_{27}H_{46}O_3$ (M—H_2O ion, *m/e* 400) and shows in its IR spectrum (Nujol) OH absorption (3350, 1055 and 1045 cm^{-1}), but no C=O absorption. On exposure to Ac_2O in pyridine at room temperature (12 hr), it furnishes a diacetate (m.p. 179–181°), $C_{31}H_{50}O_5$ (M—AcOH ion, *m/e* 442), showing in its IR (Nujol) spectrum OH absorption (3590 and 1045 cm^{-1}) besides the expected AcO absorptions (1745, 1742, 1255 and 1235 cm^{-1}). Thus, guggulsterol-II is a triol having possibly one tert-OH.

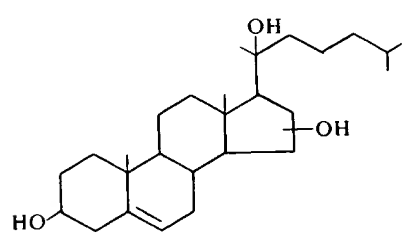
The compound is sparingly soluble in $CHCl_3$, hence its PMR spectrum was investigated in pyridine showing an $\underline{Me}_2CH$— (6H, d, 0·86 ppm, J = 6 Hz) and three $\underline{Me}$—C— (3H, s, 1·07 ppm; 6H, s, 1·44 ppm). The PMR spectrum (CCl_4) of the diacetate displays besides $\underline{Me}_2CH$— (6H, d, 0·93 ppm, J = 6 Hz), three $\underline{Me}$—C—

(3H singlets at 1·1, 1·8 and 1·25 ppm) and two $C\underline{H}_3COO$ (3H singlets at 1·96 and 2·04 ppm) absorptions, signals for two —$C\underline{H}OAc$ (two ill-defined 1H multiplets centred at 4·43 and 5·13 ppm) and one olefinic H (an ill-defined triplet at 5·31 ppm).

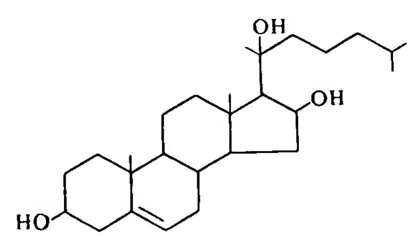
All these features are consistent with guggulsterol-II being a C_{27} steroid having two secondary and one tertiary OH, the latter being placed at C-20 to account for the total number of $\underline{Me}-\overset{|}{\underset{|}{C}}-$ groups.

Oxidation of guggulsterol-II with CrO_3 in a two phase system[25] proved complex and only one compound could be obtained TLC pure, though as a gum and in a poor yield. This product shows: λ_{max}^{EtOH} 248 nm (ε, 15,470); IR (CCl_4), OH (3490 cm^{-1}), C=O (1730 and 1690 cm^{-1}). These results are interpreted in favour of structure IX for guggulsterol-II. Mass spectral fragmentation of the triol as well as that of the diacetate are fully consistent with this and the most important fragmentations are depicted in XI–XIII; the *m/e* 254 is an important ion in the mass spectrum of the acetate and appears to arise from the M-2 AcOH ion by the fragmentation shown in XIII.

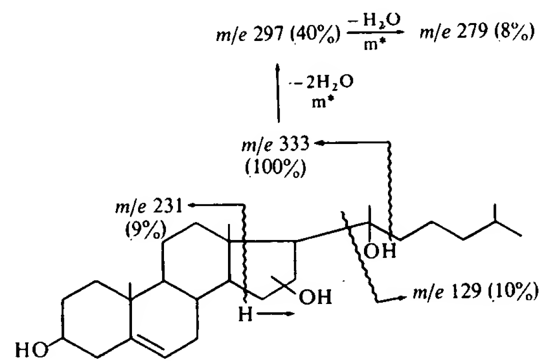
In view of the structure of guggulsterol-I, the D-ring sec OH is placed at C-16 in preference to the C-15. This speculation (X) has received full support from a partial synthesis of guggulsterol-II, which is described below:



IX

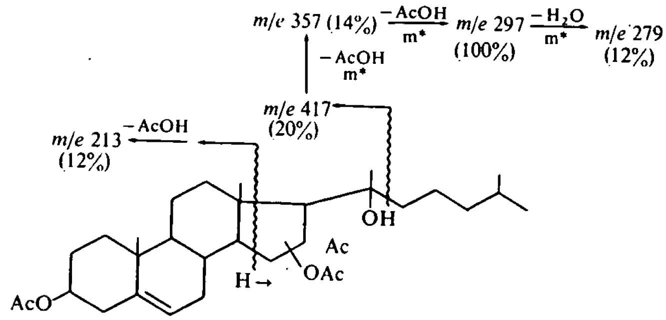


X

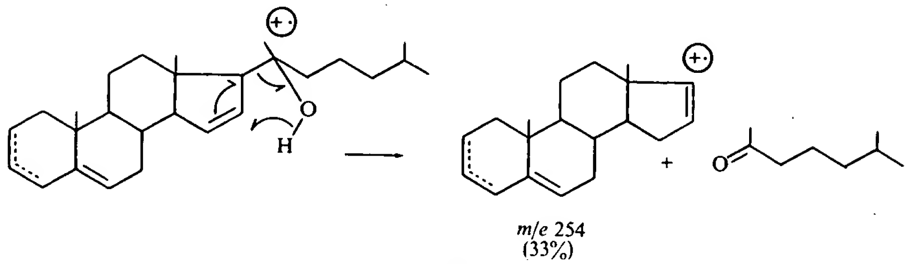


XI

The keto ester XIV available from pseudo-diosgenin diacetate[26] by CrO_3 oxidation[27] was reacted with isohexyl magnesium bromide (10 mole equiv.) in refluxing benzene to furnish a product which after saponification and chromatography

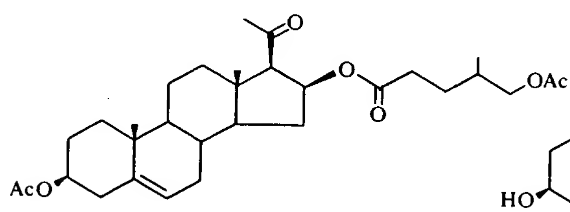


XII

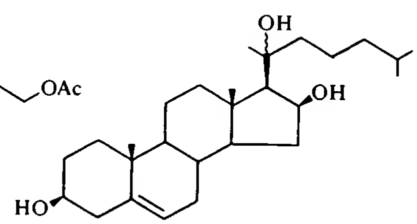


XIII

afforded XV, indistinguishable (m.p., m.m.p., [α], and m.p., m.m.p., IR, PMR spectra of the derived diacetate) from guggulsterol-II. This partial synthesis from diosgenin not only confirms the formulation X for this sterol, but also clarifies the stereochemistry at various centres (except C-20) as depicted in XV.



XIV



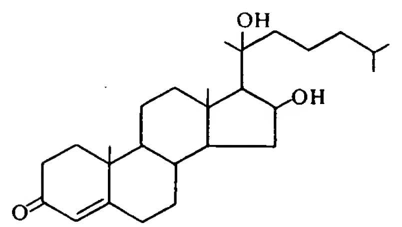
XV

Guggulsterol-III analyses for $C_{27}H_{44}O_3$ (M—H_2O ion, *m/e* 398) and displays the following structural features: OH (IR in $CHCl_3$: 3400 cm^{-1}), —CO—CH=C— (λ_{max}^{EtOH} 241 nm; ε, 17,140. IR: 1667, 1620 cm^{-1}. PMR in $CDCl_3$: 1H, s, 5·73 ppm), C<u>H</u>OH (PMR: 1H, m, 4·63 ppm), <u>Me</u>$_2$CH—(PMR: 6H, d, 0·88 ppm, J = 6 Hz) and three <u>Me</u>—C— (PMR: 6H, s, 1·21 ppm; 3H, s, 1·28 ppm). In view of the structures established for guggulsterol-I (VIII) and guggulsterol-II (XV) and the structural information outlined above, guggulsterol-III is considered to be XVI. This structure is fully supported by its electron impact induced fragmentation as depicted in XVII;

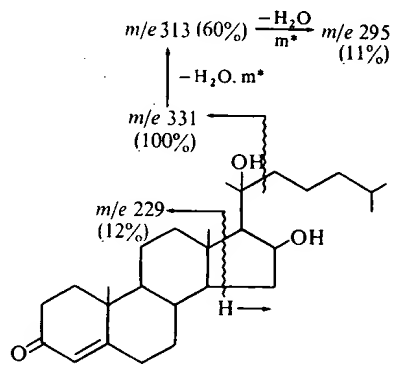
fragmentation similar to that shown in XIII (for guggulsterol-II) gives in this case an ion at *m/e* 270 (22%).

Biogenetic pattern

It has been demonstrated[28] that in mammalian tissues progesterone arises by the pathway: cholesterol → 20α-hydroxycholesterol → 20α,22S-dihydroxycholesterol → pregneneolone → progesterone. The catabolism of C_{27} precursor to C_{21} steroids in the plants is considered[29] to essentially follow the same route. The various steroids



XVI



XVII

now shown to co-occur in *Commiphora mukul* provide a satisfying biogenetic pattern fully consistent with the biosynthetic scheme. In this connection it will be pertinent to find out the C-20 stereochemistry in guggulsterol-II and guggulsterol-III and, the C-20, C-22 stereochemistry in guggulsterol-I. Due to the complexity of the gum-resin, it is inevitable that some other steroid components should have gone undetected, but it will be worthwhile from a biogenetic point of view, to look for components without oxygenation at C-16 and it is proposed to carry out this work. The occurrence in nature of 4,17(20)-pregnadiene-3,16-diones (I, II) is most interesting and a consideration of their genesis in nature offers several possibilities and if 16β-hydroxyprogesterone (guggulsterol-I → 16β-hydroxyprogesterone → *Z*- and *E*-guggulsterone) is an intermediate, its transformation into guggulsterones (I, II) calls for an interesting sequence of reactions.

EXPERIMENTAL

All m.p's are uncorrected. Light petroleum refers to the fraction b.p. 40–60°. Optical rotations were measured in $CHCl_3$.

The silica gel for column chromatography was 100–200 mesh, was washed with hot distilled water till sulphate-free, dried and activated at 125–130° (6–8 hr) and then standardized.[30] $AgNO_3$-impregnated silica gel was made by the method of Gupta and Dev[31] and activated at 100–110° (4 hr). TLC was carried out on silica gel or silica gel–$AgNO_3$ (15% $AgNO_3$) layers (0·3 mm) containing 15% gypsum.

Following instruments were used for spectral data: Perkin–Elmer spectrophotometer, model 350 (UV); Perkin–Elmer Infracord, model 137E (IR); Varian Associates A-60 spectrometer (PMR; TMS as internal standard); CEC mass spectrometer, model 21-110B (Mass; 70 eV, direct inlet system).

Broad separation

The gum-resin was in the form of light to dark brown conglomerates of tears and was only slightly sticky

to touch and had a faint balsamic odour. The material was collected from Bhuj (Gujarat), India, during September 1969.*

The gum-resin (200 g) was repeatedly triturated with light petroleum (1 lit. × 5) to yield, after solvent removal, an extract (21·5 g, thick yellow liquid) and a residue. The residue was further triturated with EtOAc (500 ml × 6) to furnish, after solvent distillation, a dark brown gum (65 g) and an insoluble residue (114 g, off-white powder).

The EtOAc-insoluble residue has high ash content (~15%) which was found† to consist of oxides of Si, Ca, Al, Mg and Fe (chemical analysis) and traces of Ti, Cu and Na oxides (AC emission flame spectroscopy).

Separation of components of light petroleum fraction

The above light petroleum extract (39 g) was chromatographed on SiO_2-gel/IIb (114 cm × 4·5 cm) to effect broad separation.

TABLE 1. BROAD-CUT SEPARATION OF LIGHT PETROLEUM EXTRACT

Fr. 1	Light pet.	1 litre × 3	3·9 g, liquid
Fr. 2	C_6H_6	1 litre × 5	14·9 g, thick liquid
Fr. 3	Ether	1 litre × 4	19·0 g, brown gum
Fr. 4	MeOH	1 litre × 2	0·5 g, rejected

Diterpene hydrocarbon. Fr. 1 above, on TLC on $AgNO_3$–silica gel (solvent: 10% ether in light petroleum) showed the presence of at least 3 compounds of which one was major. Fr. 1 (15 g) was chromatographed on $AgNO_3$–silica gel (106 cm × 4 cm) while monitoring by TLC:

Fr. 1A	light pet.	250 ml × 8	1·94 g, mixture
	10% C_6H_6 in light pet.	250 ml × 6	
Fr. 1B	C_6H_6	250 ml × 2	1·93 g, mixture
Fr. 1C	C_6H_6	250 ml × 10	12·0 g, essentially single spot
	2% EtOAc in C_6H_6	250 ml × 5	
Fr. 1D	EtOAc	250 ml × 4	0·8 g, mixture

Fr. 1C (2·0 g) was rechromatographed on $AgNO_3$–silica gel (40 cm × 1·2 cm) using 10% ether in light petroleum as the eluent to furnish a TLC pure liquid (1·6 g), b.p. 150–152°/0·8 mm, n_D^{30} 1·5102, $[\alpha]_D$ −19·7° (*c*, 0·35%) (Found: C, 88·21; H, 12·11. $C_{20}H_{32}$ requires: C, 88·16; H, 11·84%).

Diterpene alcohol. Fraction 2 (Table 1) was found to be a mixture of at least 5 compounds, one of which was major (TLC; 30% ether in light petroleum). This mixture (5 g) was chromatographed on SiO_2–gel/IIa (100 cm × 4 cm) and followed up by TLC:

Fr. 2A	light pet.	1 lit. × 3	0·22 g, gum, mixture
	50% C_6H_6 in light pet.	500 ml × 6	
Fr. 2B	C_6H_6	250 ml × 1	0·29 g, gum, mixture
Fr. 2C	C_6H_6	200 ml × 3	3·35 g, gum, single
Fr. 2D	C_6H_6	250 ml × 5	0·67 g, gum, mixture
Fr. 2E	EtOH	250 ml × 4	0·32 g, gum, mixture

Fr. 2C (1·16 g) was crystallized from MeCN (1 ml) at ~ −10° and the solid (0·70 g) separated by inverse filtration and further recrystallized from MeCN, m.p. 37–38°, $[\alpha]_D$ +53° (*c*, 0·47%) (Found: C, 82·88; H, 11·81. $C_{20}H_{34}O$ requires: C, 82·69; H, 11·80%).

(+)-*Sesamin.* Fr. 3 (Table 1) was a very complex mixture by TLC (60% ether in light petroleum) and was

* The authors are grateful to Dr. C. K. Atal and Dr. C. Dwarakanath for the supply of raw material.
† The analysis was kindly carried out by Dr. P. R. Subbaraman and the authors are grateful to him for this help.

chromatographed on SiO_2-gel/IIb (124 cm × 4·5 cm) using increasing amounts of EtOAc in C_6H_6:

Fr. 3A	2% EtOAc in C_6H_6	500 ml × 3	2·11 g, gum, mixture
Fr. 3B	5% EtOAc in C_6H_6	250 ml × 6	2·64 g, gum, mixture
Fr. 3C	EtOAc in C_6H_6	250 ml × 7	3·28 g, gum, mixture
Fr. 3D	EtOAc in C_6H_6	250 ml × 14	4·52 g, gum, mixture
Fr. 3E	EtOAc in C_6H_6	250 ml × 8	1·15 g, gum, mixture
Fr. 3F	EtOAc in C_6H_6	250 ml × 12	1·60 g, gum, mixture
Fr. 3G	10% EtOAc in C_6H_6 25% EtOAc in C_6H_6	250 ml × 5 250 ml × 14	2·92 g, gum, mixture
Fr. 3H	EtOH	250 ml × 8	1·40 g, gum, mixture

Fr. 3A (2·11 g) was rechromatographed on SiO_2-gel/IIb (120 × 2 cm) and eluted with increasing amounts of ether in light petroleum. Fractions eluted with 20% ether in light petroleum were combined (0·56 g), dissolved in MeOH and chilled to furnish a colourless crystalline solid (160 mg), m.p. 120–123°, $[\alpha]_D$ +53·3° (*c*, 1·8%), identified as sesamin by comparison (m.m.p., IR) with an authentic sample (Lit.[15]: m.p. 123°, $[\alpha]_D$ 71°).

Cholesterol. Fr. 3B (2·64 g) was rechromatographed on SiO_2-gel/IIb (100 cm × 2 cm) as above and the material (1·56 g) eluted with 30% ether in light petroleum, on treatment with MeOH gave a solid (0·75 g), which after several recrystallizations from MeOH furnished white flakes m.p. 139–142°, identified as cholesterol by comparison (m.m.p., IR, PMR) with an authentic sample.

Z-Guggulsterone [4,17(20)-trans-*pregnadiene*-3,16-*dione*]. Fr. 3D (4·52 g), on treatment with ether and chilling gave a crystalline solid (1·44 g; m.p. 187–191°). Its mother liquor and Fr. 3E were combined (3·7 g) and chromatographed on SiO_2-gel/IIb (105 cm × 2·7 cm) using increasing amounts of ether in light petroleum as eluent with TLC monitoring (60% ether in light petroleum). The material (1·07 g), on treatment with ether as before furnished an additional quantity (0·38 g; m.p. 182–189°) of the same solid (TLC). The combined solids were recrystallized from acetone to furnish colourless prisms (1·23 g), m.p. 192–193°, $[\alpha]_D$ −77·1° (*c*, 2·07%); λ_{max}^{EtOH} 241 nm (ε, 27,100). IR (Nujol): C═O 1720, 1675 cm^{-1}; C═C 1650, 1620 cm^{-1}. PMR ($CDCl_3$): C-18 Me (3H, s, 0·97 ppm), C-19 Me (3H, s, 1·23 ppm), C-21 Me (3H, d, 2·08 ppm, J = 7 Hz), C-4H (1H, b s, 5·75 ppm) and C-20H (1H, q, 5·73 ppm, J = 7 Hz). Mass spectrum: important ions at *m/e* 312 (M^+, 41%), 298 (24%), 297 (100%), 135 (13%), 121 (8%), 105 (9%), 93 (10%), 91 (18%), 79 (14%), 77 (12%), 55 (9%) and 53 (9%). (Found: C, 80·61; H, 9·01. $C_{21}H_{28}O_2$ requires: C, 80·73; H, 9·03%) [Lit.[18]: m.p. 188–190°; $[\alpha]_D$ −61°; λ_{max} 241 nm (ε 25,000); IR; PMR].

A soln of *Z*-guggulsterone (0·30 g) in THF (20 ml) was added dropwise to a soln of Li (650 mg) in liquid NH_3 (180 ml) during 10 min with continuous stirring. After stirring for another 90 min excess of Li was destroyed by NH_4Cl (13 g), NH_3 evaporated, water (75 ml) added and the product taken up in $CHCl_3$ (50 ml × 3). Solvent removal yielded a gum (0·31 g) which was dissolved in pyridine (3·5 ml) and oxidized with Sarett reagent (CrO_3, 320 mg; pyridine, 3·5 ml) for 16 hr at ~25° to furnish after usual work up the crude saturated ketone (268 mg) which was purified by PLC (25% EtOAc in C_6H_6) to give after crystallization from aq. MeOH, 5α-pregnan-3,16-dione (146 mg), m.p. 125–127°; IR ($CHCl_3$): C═O 1740, 1715 cm^{-1} (Lit.[19]: m.p. 124–128°).

E-*Guggulsterone* [4,17(20)-cis-*pregnadiene*-3,16-*dione*]. Fr. 3F (1·60 g) on treatment with ether and chilling furnished a solid (0·25 g) which, on further recrystallization (C_6H_6–light petroleum) at −10° gave colourless needles (TLC pure; solvent: 60% ether in light petroleum), m.p. 168–170°, $[\alpha]_D$ −28·4° (*c*, 2·11%), λ_{max}^{EtOH}, 241 nm (ε, 22,220). IR (Nujol): C═O 1720, 1670 cm^{-1}; C═C 1650, 1620 cm^{-1}. PMR (CCl_4): C-18 Me (3H, s, 1·07 ppm), C-19 Me (3H, s, 1·23 ppm), C-21 Me (3H, d, 1·85 ppm, J = 7 Hz), C-4H (1H, b s, 5·67 ppm) and C-20H (1H, q, 6·45 ppm, J = 7 Hz). Mass spectrum: important ions at *m/e* 312 (M^+ 100%), 298 (10%), 297 (41%), 271 (10%), 270 (42%), 255 (13%), 227 (8%) and 214 (8%). (Found: C, 80·71; H, 9·12. $C_{21}H_{28}O_2$ requires: C, 80·73; H, 9·03%). [Lit.[18]: m.p. 170–171·5°; $[\alpha]_D$ −30°; λ_{max} 241 nm (ε, 27,600); IR; PMR].

Saponification and chromatography of EtOAc *extract*

The EtOAc extract (100 g) was refluxed (N_2) with 10% aq. methanolic KOH (2·0 lit.) for 3 hr. Water (500 ml) was added and MeOH (~400 ml) removed: after further dilution with H_2O (1·51 l) and usual work up with ether, acidic material (41·5 g) and non-saponifiable portion (56 g, reddish gum) were obtained. The latter was chromatographed on SiO_2-gel/IIb (120 cm × 7 cm) using increasing amounts of EtOAc

in C_6H_6 as the eluent and with TLC monitoring [solvents: (a) 60% ether in light petroleum: (b) 10% MeOH in C_6H_6]:

Fr. 1	C_6H_6	500 ml × 10	1·30 g, gum, mixture
Fr. 2	2% EtOAc in C_6H_6	500 ml × 5	3·25 g, gum, mixture
Fr. 3	2–5% EtOAc in C_6H_6	500 ml × 9	2·04 g, gum, mixture
Fr. 4	10% EtOAc in C_6H_6	500 ml × 8	2·11 g, gummy crystalline mixture
Fr. 5	25% EtOAc in C_6H_6	500 ml × 5	0·91 g, gum, mixture
Fr. 6	25% EtOAc in C_6H_6	500 ml × 2	3·75 g, gummy crystalline mixture
Fr. 7	25% EtOAc in C_6H_6	500 ml × 2	2·19 g, gum, mixture
Fr. 8	25–50% EtOAc in C_6H_6	500 ml × 4	2·91 g, gummy crystalline mixture
Fr. 9	50% EtOAc in C_6H_6	500 ml × 4	4·77 g, gum, mixture
Fr. 10	50% EtOAc in C_6H_6	500 ml × 4	1·49 g, gummy crystalline mixture
Fr. 11	50% EtOAc in C_6H_6	500 ml × 3	1·54 g, gum, mixture
Fr. 12	50% EtOAc in C_6H_6	500 ml × 8	2·65 g, gummy crystalline mixture
Fr. 13	EtOAc	500 ml × 4	3·53 g, gum, mixture
Fr. 14	2–50% MeOH in EtOAc	500 ml × 28	16·39 g, gummy solid
Fr. 15	MeOH	500 ml × 4	2·25 g, rejected

Fr. 6 (3·75 g) was chromatographed on SiO_2–gel/IIb (112 cm × 2·5 cm) and eluted with increasing amounts of EtOAc in C_6H_6. Fractions eluted with 25% EtOAc in C_6H_6 were combined and crystallized from acetone to furnish *Z*-guggulsterone (0·566 g), m.p. 189–191°.

Fr. 8 (2·91 g) was similarly chromatographed on SiO_2–gel/IIb (94 cm × 2·5 cm). The fractions eluted with 25% EtOAc in C_6H_6 were combined, treated with ether and chilled to furnish *E*-guggulsterone (0·39 g) which on recrystallization from C_6H_6–light petroleum had m.p. 168–170°.

Guggulsterol-I (VIII). Fr. 12 (2·60 g) was chromatographed on SiO_2–gel/IIb (90 cm × 2·5 cm) and eluted with increasing proportions of EtOAc in C_6H_6. Fractions eluted with 50% EtOAc in C_6H_6 were combined (1·9 g), treated with MeCN (5 ml) and chilled to furnish a solid (m.p. 221–226°, 750 mg) which on recrystallization from aq. MeOH gave colourless crystals, m.p. 225–228°, $[\alpha]_D$ +77·6° (*c*, 2·01%). (Found: C, 75·28; H, 10·63. $C_{27}H_{44}O_4$ requires: C, 74·95; H, 10·25%).

A soln of guggulsterol-I (305 mg) in MeOH (15 ml) was treated with a soln of $NaIO_4$ (311 mg) in water (3 ml) and MeOH (12 ml) and kept in the dark (36 hr). The mixture was diluted with water (75 ml) and extracted with CH_2Cl_2 and the crude product chromatographed on SiO_2–gel/IIa (39 cm × 1·2 cm). The material eluted with CH_2Cl_2 (150 ml) was, after solvent removal, treated with 2,4-dinitrophenylhydrazine (H_2SO_4 method) diluted with water (10 ml) and the derivative extracted with $CHCl_3$. PLC (solvent: 25% light petroleum in C_6H_6) of this product gave 2,4-DNP of *iso*caproic aldehyde (top cut, 12 mg) m.p. 88–91°: m.m.p. with authentic sample (m.p. 88–90°) was undepressed and their spectra (IR, PMR) were identical. The chromatography fraction (165 mg) eluted with 2% MeOH in CH_2Cl_2 (50 ml) was essentially pure by TLC (10% MeOH in C_6H_6); this was further purified by PLC and the product recrystallized from acetone-light petroleum to give 16β-hydroxyprogesterone (100 mg), m.p. 203–204°, $[\alpha]_D$ +207·5° (*c*, 1·6%): λ_{max}^{EtOH} 240 nm (ε, 17,600). IR ($CHCl_3$): OH 3420, 1040 cm^{-1}; C=O 1705, 1665 cm^{-1}; C=C 1620 cm^{-1}. PMR ($CDCl_3$): 2 quaternary Me's (6H, s, 1·2 ppm), $C\underline{H}_3C{=}O$ (3H, s, 2·2 ppm), $C\underline{H}OH$ (1H, m, 4·55 ppm), $O{=}\overset{|}{C}{-}C\underline{H}{=}\underset{|}{C}{-}{-}$ (1H, s, 5·7 ppm). (Found: C, 76·61; H, 9·19. $C_{21}H_{30}O_3$ requires: C, 76·32; H, 9·15%). (Lit.[24]: m.p. 202–203°; λ_{max} 240 nm, ε, 16,500; IR; Mass).

Guggulsterol-II (XV). On treatment with MeCN fr. 10 (1·49 g) deposited a solid (70 mg) which was recrystallized from MeOH to furnish colourless crystals m.p. 231–233° (evacuated sealed capillary), $[\alpha]_D$ −42·3° (*c*, 0·22%). (Found: C, 77·32; H, 10·83. $C_{27}H_{46}O_3$ requires: C, 77·46; H, 11·08%). Its *diacetate* (Ac_2O, pyridine, room temp/12 hr) was obtained as snow-white flakes, m.p. 179–181° (MeOH). (Found: C, 73·35; H, 9·96. $C_{31}H_{50}O_5$ requires: C, 74·06; H, 10·03%).

A stirred slurry of guggulsterol-II (127 mg) in ether (100 ml) was treated with Brown's reagent[25] ($Na_2Cr_2O_7$ 1 g, conc. H_2SO_4 0·75 ml made up to 5 ml with H_2O) in portions, at 25–30°. After 2 hr, the faint-orange soln was treated with a few drops of MeOH and worked up to furnish a gum (61 mg) which was purified by PLC (solvent: 5% MeOH in C_6H_6); the required ketonic compound (highest R_f, 16 mg) was

now TLC pure but still remained a gum. PMR (CCl_4): $\underline{Me}_2CH$—(6H, d, 0·88 ppm, J = 6 Hz), 3 quaternary Me's (6H, s, 1·2 ppm; 3H, s, 1·3 ppm), O=C—C$\underline{H}$=C (1H, s, 6·1 ppm).

Guggulsterol-III (XVI). Fr. 9 (4·77 g) was chromatographed on SiO_2-gel/IIb (90 cm × 3 cm), the fractions eluted with 25% EtOAc in C_6H_6 were combined and treated with MeCN (~5 ml). The separated solid (273 mg, m.p. 160–165°) was a mixture (TLC) of two compounds one of which was guggulsterol-II. The other compound (95 mg) was isolated by PLC (10% MeOH in C_6H_6) and recrystallized from acetone to afford colourless silky needles (42 mg), m.p. 181–183°, $[\alpha]_D$ +75·3° (c, 0·17%). (Found: C, 77·78; H, 10·87. $C_{27}H_{44}O_3$ requires: C, 77·83; H, 10·65%).

Partial synthesis of guggulsterol-II

Pseudodiosgenin diacetate[26] (5·0 g, m.p. 94–97°) in gl. AcOH (100 ml), cooled to 15° in ice-water bath, was oxidized[27] with a soln of CrO_3 (3·5 g) in H_2O (3·5 ml) and AcOH (10 ml). The temp rose to 28° and after 45 min it was poured into water (300 ml) and the neutral product isolated with ether to furnish a gum (4·48 g); TLC (15% EtOAc in C_6H_6) showed it to be a complex mixture. A TLC pure fraction (1·51 g) was isolated by IDCC[32] on SiO_2-gel (25 cm × 9·4 cm; solvent 15% EtOAc in C_6H_6) and recrystallized from MeOH to furnish colourless crystals of ketoester XIV (817 mg), m.p. 82–85° (Lit.[27]: m.p. 84–86°).

Mg turnings (632 mg) in dry ether (20 ml) were stirred and treated with isohexyl bromide[33] (3·33 g) in dry ether (30 ml) in a 3-necked flask equipped with a dropping funnel, stirrer and condenser and also provided with a gas-inlet tube for passing O_2-free dry N_2. After 20 min stirring at room temp the mixture was gently refluxed (3 hr) on a waterbath. To this Grignard reagent (estimated:[34] 60%) was added the keto ester XIV (521 mg) in dry ether (10 ml) and refluxed for 1 hr with stirring. Benzene[35] (50 ml) was added and the ether distilled off and the resulting product further refluxed for 14 hr. The complex was decomposed with NH_4Cl aq., C_6H_6 layer separated, aq. portion extracted with ether and the combined organic portions washed, dried and evaporated to furnish a material, which was a complex mixture (TLC: solvent 10% MeOH in C_6H_6), but had a component with the same R_f as guggulsterol-II. The products from two such experiments were combined, hydrolyzed with aq. ethanolic KOH (10%, 42 ml) at reflux (4 hr, N_2) and the crude material (2·0 g) treated with C_6H_6–ether when cryst. guggulsterol-II (129 mg) separated. The crude material from the filtrate was chromatographed on SiO_2-gel/IIb (110 cm × 2·5 cm) and eluted with C_6H_6 followed by increasing amounts of EtOAc in C_6H_6. The fraction eluted with 25% EtOAc in C_6H_6 on treatment with MeCN yielded an additional quantity of guggulsterol-II (34 mg). The combined products were recrystallized from MeOH to furnish pure guggulsterol-II (68 mg), m.p. 231–234° (evacuated sealed capillary), m.m.p. with natural sample (m.p. 231–233°) was undepressed: $[\alpha]_D$ −45·2° (c, 0·2%). *Diacetate*, m.p. 177–179°; m.m.p. with authentic guggulsterol-II diacetate (m.p. 179–181°) was undepressed.

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ORGN . . .
ANEMIA, EDEMA, SALIVATION AND HEAVINESS OFTHE STOMACH. POTION MIXED WITH URINE. BOERHAAVIA VERTICILLATA. BERBERIS ARISTATA, TERMINALIA CHEBULA, TINOSPORA CORDIFOLIA, AND **COMMIPHORA MUKUL**.

ORGN Class: DICOT Family: EUPHORBIACEAE Genus: PHYLLANTHUS Species: EMBLICA
Synonym(s): EMBLICA OFFICINALIS
Organism part: DRIED PART NOT SPECIFIED
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Extract type: DECOCTION
Dosage Information: ORAL; HUMAN ADULT
Comment(s): USED FOR **DIABETES**. DECOCTION MIXED WITH HONEY.

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ORGN Class: DICOT Family: COMBRETACEAE Genus: TERMINALIA Species: CHEBULA
Organism part: DRIED FRUIT
Geographic area. . .
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Extract type: DECOCTION
Dosage Information: ORAL; HUMAN ADULT
Comment(s): USED FOR **DIABETES**. DECOCTION MIXED WTIH HONEY.

ORGN Class: DICOT Family: CUCURBITACEAE Genus: CITRULLUS Species: COLOCYNTHIS
Organism part: DRIED PART NOT SPECIFIED. . .
OF STUDY (STY): FOLKLORE Classification (CC): ANTIHYPERGLYCEMIC ACTIVITY
Extract type: DECOCTION
Dosage Information: ORAL; HUMAN ADULT
Comment(s): USED FOR **DIABETES**. DECOCTION MIXED WITH HONEY.

ORGN Class: DICOT Family: NYCTAGINACEAE Genus: BOERHAVIA Species: VERTICILLATA
Organism part: DRIED PART NOT SPECIFIED. . .
ANEMIA, EDEMA, SALIVATION AND HEAVINESS OFTHE STOMACH. POTION MIXED WITH URINE, BOERHAAVIA VERTICILLATA, BERBERIS ARISTATA, TERMINALIA CHEBULA, TINOSPORA CORDIFOLIA, AND **COMMIPHORA MUKUL**.

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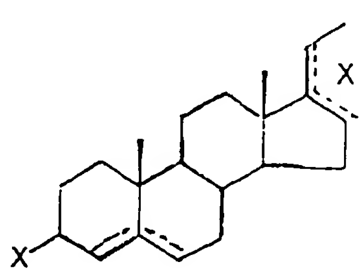
(54) Medicaments for hypolipidemic and hypoglycemic conditions

(57) The invention provides a method of using pregnadienones and pregnadienols represented by the structural formula (I) as shown herein below



I

Wherein X=OH or O or combination thereof and positioning of olefinic bonds are at 4(5); 5(6); 6(17); 17(20) or various combinations and said compounds containing at least one olefinic bond in or on their D-ring for the treatment of hypolipidemic and hypoglycemic conditions in mammals, said method comprising administering an effective amount of the said compounds to the recipient mammals.

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Description

Field of the invention

[0001] This invention relates to the novel use of D-ring unsaturated pregnadienols/ pregnadienones represented by general formula I as shown in the accompanying drawings, possessing both pronounced hypolipidemic and hypoglycemic activities and devoid of androgenic and progestational activities. More particularly this invention relates to the novel use of 3β-hydroxy-pregna-5, 16-dienone an important prototype of this class, represented by the formula (II) as shown in the accompanying drawings, for the treatment of diabetes and pronounced hypolipidemic and hypoglycemic activities.

Background

[0002] High plasma cholesterol and related lipids are known to be one of the factors that predispose an individual to atherosclerosis and thus to myocardial infraction. Diabetes mellitus, which eventually impairs the function of kidneys, eyes, nervous and vascular systems, is quite often associated with lipid disorders. Both hyperlipidemia and diabetes mellitus require long term management and pose problems in choice of pharmacotherapeutic interventions when these conditions manifest together. Though a number of drugs are known separately to treat these conditions, there are a number of side effects associated with them which limit their long term use.

[0003] The most important hypolipidemic drugs available today belong to the statin and fibrate classes [McCarthy, P.A., Med. Res. Rev., 13, 139-59 (1993)] whereas hypoglycemic drugs fall into the category of sulphonylureas, biguanidines and amidines [Wolff, M.E. (Ed), Burger's Medicinal Chemistry Part II, 1045 (1981), John Wiley & Sons, New York]. However, these therapeutic agents are not free of side effects-statins (HMG-CoA reductase inhibitors) the most widely used drugs today which hitherto were thought to be very safe drugs, have exhibited side effects following long term therapy [Carrier, M. et al.; Ann. Thorac. Surg., 57, 353-6 (1994)]. The adverse effects which have become the source of concern, are increases in hepatic transaminases and myopathies [Witztum, J.L., In Goodman & Gilman's The Pharmacological Basis of Therapeutics, eds. Hardman, J. et al., 9th edition, McGraw Hill, New York pp. 875-98, Fukami, M. et al; Res. Exp. Med., 193, 263-73 (1993); Appelkvist, E..; et al.; Clin. Invest., 71 (suppl 8), 597-102 (1993), Wills, R.A. et al.; Proc. Natl. Acad. Sci. (US), 87, 8928-30 (1990)] and carcinogenesis specially breast cancer in subjects undergoing treatment with pravastatin [Braunwald, E.; Scrip, 2117, 33 (1996)); Ciaravino, V. et al.; Mutat. Res.; 353, 95-107 (1995)]. The incidence of myopathy associated with rhabdomyolysis and renal failure is increased subsequent to such treatment [East, C. et al.; N. Engl. J. Med., 318, 47-48 (1998); Pierce L.R. et al.; J. Am.Med. Assoc., 265, 71-75 (1990)]. Also, these HMG-CoA inhibitors block mevalonate production which occurs at an early stage in cholesterol synthetic pathway. Mevalonate is a common precursor for all isoprenoids such as ubiquinones (Co-enzyme Q-10), the dolichols, isopentenyl t-RNA etc. Therefore, long term blockade of mevalonate synthesis leads to Q-10 deficiency. Serum Co-enzyme Q-10 is important for cardiac function {Laaksonen, R. et al., Eur. J. Clin. Pharmacol. 46,313-7 (1994); Bargossi, A.M. et al; Int.J. Clin. Lab. Res., 24, 171-6 (1994)]. The commonest side-effects of fibrates and particularly clofibrate therapy are gastrointestinal upsets including nausea, vomiting, diarrhoea, dyspepsia, flatulence and abdominal discomfort [Oliver ,M. F. et al.; Br. Heart J., 40, 1069-1118 (1978)]. Elevated creatine phosphokinase concentration during clofibrate therapy may be associated with a syndrome of muscle pain and weakness. Large-scale long-term studies have demonstrated an increased incidence of cholecystitis, gallstones and sometimes pancreatitis in patients receiving clofibrate and some studies have indicated cardiovascular disorders [The coronary Drug Project Research Group; N. Engl. J. Med., **296**, 1185-90 (1977)]. The unexpected finding of an increased mortality rate in patients taking clofibrate in the WHO study produced serious concem over the long-term safely of clofibrate and ultimately led to its withdrawal in many countries [Oliver, M. F. et al ; Lancet, **ii**, 600-604 (1984)].

[0004] The adverse effects of biguanidine antidiabetic agents include gastro-intestinal disturbances like diarrhoea and lactic acidosis [Paterson, K. R. et al.; Adverse Drug React Acute Poisoning Rev., 3, 173-82 (1984)]. With sulphonylureas the commonly associated adverse effects are hypoglycemia, gastrointestinal disturbances, hypersensitivity and vascular complications [Paice, B.J. et al., Adverse Drug React. Acute Poisoning, **4**, 23-26 (1985)]. As diabetes and hyperlipidemia are quite commonly manifesting together, it would be of great clinical benefit if the same compound could have both these activities together because the available drugs are not free of toxic effects and neither data regarding toxic manifestations are available when drugs for two clinical conditions are mixed together.

[0005] Two approaches currently being pursued in search of drugs with hypolipidemic and hypoglycemic activities together. The first approach emerged during detailed study of antihypertensive action of adrenergic receptor modulators. The study revealed that α_1-adrenergic blockers (particularly Doxazosin and Prazosin) [Lithell, H.O. ; J. Hypertens, **15** (Suppl 1), S 39-42 (1997); Poliare,T. et al.; Diabetologia, **31**, 415-420 (1988); Anderson, P.E. et al.; Am. J. Hypertens, 9, 323-333 (1996)] and β_3-adrenergic agonist (BTA-243, BRL-37344, CGP 12177, CL 316243 [Arch, J. R. S. et al.; Med. Res. Rev., **13**, 663-729 (1993); Largis, E. E. et al;. Drug Dev. Res.. **32**, 69-76 (1994)] also affect plasma lipoprotein

metabolism and increase insulin sensitivity; As a result such antihypertensive drugs exhibit lipid lowering and hypoglycemic actions together, α_1-adrenergic receptor blockers, however have the inherent limitations of causing orthostatic hypotension and syncope [Matyus, P.; Med. Res. Rev., **17**(6), 523-35 (1997)]. The essential requirement of β_3-agonist for antiobesity and antidiabetic actions is the need for high selectivity for β_3-adrenoceptor, Any substantial β_1- or β_2-agonism would likely cause increased heart rate and muscle tremor respectively which are unacceptable in a drug which could be administered on long term basis [Connacher, A . A. et al.; Brit. Med. J., **296**, 1217-20 (1988); Mitchell, T. H. et al; Int. J. Obesity., **13**(6), 757-66 (1989)]. The second line of approach for dual activity came into light during the study of anti-oxidant property of drugs. There have been many reports describing relationships between peroxidation and diseases such as diabetes mellitus, atherosclerosis and myocardial ischemia in terms of radical oxidation. Troglitazone, an antioxidant drug has been developed as an oral hypoglycemic agent which enhances the action of insulin in peripheral tissues and liver besides its hypolipidemic effects. However, troglitazone is also not free of major side effect causing liver damage. The drug, troglitazone, has been implicated in 35 cases of liver disease leading to one transplant and one death [Warner-Lambert; Chem. & Ind., No.**22.** 897 (1997)]. Thus to the best of our knowledge no class of compound is yet available which has both effects together as the main action and have fair safety margin.

[0006] We, in early eighties started our work for search of such compounds which have effect on endogenous transportation of lipids and glucose rather than interfering with exogenous transportation. Our research was mainly based on secondary metabolic actions of progesterone.

[0007] Progesterone, apart from its classical hormonal action on the reproductive system, is known to modulate lipid, carbohydrate, insulin and protein metabolism. The rise in the level of progesterone in the first trimester of pregnancy causes hyperphagia, pancreatic islet hypertrophy, hyperinsulinemia and body fat and glycogen deposition, when the metabolic demands of the fetus are very low. However, in the latter half of pregnancy, although the progesterone levels are still high, the carbohydrate, lipid and protein reservoirs shift into circulation to meet the needs of the growing fetus. [Kalkhoff, R.K.; Am. J. Obstet. Gynecol., **142**, 735-38 (1982)].

[0008] Progesterone thus, having actions both on the reproductive and the metabolic systems, seemed to offer the possibility of dissociating these two biological activities by structural modifications. The experience of the development of second generation progestins supported this contention. The first generation progestins such as levonorgestrel exhibited undesirable pharmacologic effects like alteration in carbohydrate and lipoprotein metabolism, weight gain and hypertension, which was shown to be related to their intrinsic androgenic/anabolic activity and ability to bind with androgen receptors. The androgenic affinity has been attributed to C-17 hydroxy functionality which makes these molecules resemble androgens. In recently discovered second generation progestins such as gestodene and 3-keto-desogestrel, an additional olefinic bond either in C- or D- ring brought a dramatic decrease in their affinity to androgen receptors (Table 1). As a result these compounds have a very high order of progestational effect with practically no androgenic activity and did not cause hyperlipidemia [London, R.S.; Obstetrical & Gynocological Survey, **47**, 777-81 (1992)].

Table 1.

Relative Binding Affinity of Contraceptive Progestins for Progesterone and Androgen receptors			
	Progestin Receptor Binding Affinity	**Androgen Receptor Binding Affinity**	**Selectivity Index* (A/P ratio)**
Progesterone	1.00	0.005	93
Levonorgestrel	5.41	0.220	11
3-Keto-desogestrel	8.6	0.120	33
Gestodene	9.21	0.154	28

* The higher the selectivity index, the greater the separation between the dose needed to achieve the desired progestational effect and the dose associated with the undesired androgenic effect [Collins, D.C. Am. J. Obstet. Gynecol. 170, 1508-13 (1994)].

Objects of the Invention

[0009] It is an object of the invention to explore the possibility of designing pregnadienones which while preserving the ability to modulate lipid and carbohydrate metabolism would not have any progestational effect. It would be pertinent to point out that earlier, the applicants had isolated a D-ring modified pregnenolone, named Gugulsterone represented by formula (9) as shown in Table 2, from guggul resin obtained from *Commiphora mukul,* which had potent hypolipidemic effect without any progestational effect [Arya, V.P.; Drugs Fut. 13, 618 (1998)].

[0010] It is another object of the invention to explore the possibility of dissociating the hypolipidemic and insulin sensitizing activities of progesterone from its hormonal actions. Accordingly, the applicants focussed their attention to

prepare and investigate analogues/prototypes with additional substituents in ring-D of pregnadienones.

Brief description of the accompanying drawings

[0011]

Figure 1 (I) represents the structural formula of compounds belonging to the class of pregnadienones and pregnadienols and

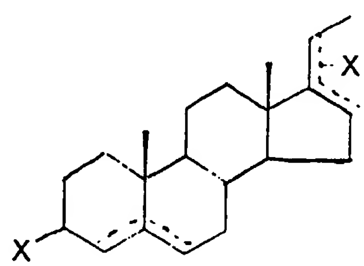
Figure 1 (II) represents the structural formula of 3β-hydroxypregna-5, 16-lien-20-one

Figure 2 represents the structural formulae of hormones.

Summary of the Invention

[0012] In accordance with the above objectives, the applicant's present invention relates to a method of using D-ring unsaturated pregnadienones represented by structural formula I which causes significant fall of serum cholesterol, triglycerides, LDL-cholesterol and glucose with mild increase in HDL-cholesterol, said method comprising administration of effective amounts of said compounds of formula (I) to mammals. The compounds possess fair safety margin having antioxidant and cardio protection activities.

[0013] The invention also provides a method of treatment of hypolipidemic and hypoglycemic conditions which comprises administration to a recipient a therapeutic composition comprising a pharmaceutically effective amount of compound D-ring unsaturated preganadienones represented by the general formula I as shown hereinbelow and in the accompanying drawings:



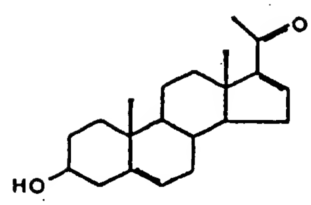
I

Wherein X= OH or O or combination thereof and positioning of olefinic bonds are at 4(5); 5(6); 16(17); 17(20) or various combinations

Detailed description of the invention

[0014] The present invention concerns methods for lowering serum cholesterol, triglycerides and glucose levels in subjects with obesity and diabetic conditions or prophylactically holding in check the symptoms of such a disease state.

[0015] In particular, the applicants, during their study, have observed that the pregnadienone, 3β-hydroxypregna-5, 16-dien-20-one represented by the structural formula (II) shown hereinbelow and in the accompanying drawings is useful for the treatment of hypolipidemic and hypoglycemic conditions.



II

[0016] Accordingly, the invention provides a method of using compounds represented by the structural formula (I) as shown in the accompanying drawings, containing at least one olefinic bond in or on their D-ring for the treatment of hypolipidemic and hypoglycemic conditions in mammals, said method comprising administering an effective amount of the said compounds to recipient mammals.

[0017] In one embodiment, the compounds of formula (I) are administered in the form of tablets, capsules or injectibles.

[0018] In another embodiment, the compounds of formula (I) are characterised as pregnadienones and pregnadienols.

[0019] In yet another embodiment, the most preferred compound belonging to the family of preganienones and pregnadienols represented by formula (I) is 3β-hydroxy-pregna-5,16-dien-20-one, which is represented by the structural formula (II) as shown in the accompanying drawings.

[0020] In a further embodiment, the compounds of formula (I) are optionally administered to the recipient mammal as an admixture with conventional anti-platelet, anti artherosclerotic, hypolipoproteinic and antidiabetic drugs.

[0021] In still another embodiment, the compounds of formula (I) are essentially free of side effects associated with conventional hypolipidemic and hypoglycemic drugs.

[0022] In an embodiment, the compounds of formula (I) exhibit cardioprotective, anti-diabetic, anti-artherosclerotic and anti-oxidant properties.

[0023] Further, the invention provides a method of treatment of hypolipidemic and hypoglycemic conditions in mammals, which comprises administration to a recipient, a therapeutic composition comprising an effective amount of compound of formula (I) with conventional carriers.

[0024] In an embodiment, the recipient mammals are selected from the group comprising rats, human beings, rhesus monkeys and rabbits.

[0025] In another embodiment, the conventional carriers are selected from anti-platelet, anti-artherosclerotic, hypolipoproteinic and anti-diabetic drugs.

[0026] In yet another embodiment, the said compounds of formula (I) essentially contain an olefinic bond in or on their D-ring.

[0027] In a further embodiment, the compounds of formula (I) are essentially free of androgenic, progestinal and side effects.

[0028] In still another embodiment, the therapeutic composition is administered in the form of tablets, capsules and injectibles.

[0029] In another embodiment, the said preganadienones and preganadienols exhibit cardio protective, antidiabetic, antialtheroselerotic and antioxidant properties.

[0030] In yet another embodiment, the said preganadienones and pregnadienols of formula I essentially contain olefinic bond in one of the D-rings.

Methods of synthesis/production

[0031] 3β-Hydroxy pregna-5, 16-dien-20-one The methods of synthesis are essentially known in the literature/can be obtained from diosgenin by chemical degradation [G. Rosenleranz "History of Steroids", Steroids, 57, 409 (1992)]. Although, it was later isolated from Veratrum Grandiflorum [Kanko, K. et al; Phytochemistry, 12 1509 (1973)] but yield is too low to be of any practical value. Oppenauer oxidation of **2** with aluminium-*iso*propoxide and cyclohexanone in toluene produces 4,16-dienpregna-3,20-dione [16-dehydroprogesterone, (**3**)]. The C-16(17)olefinic bond in **1** is selectively reduced with Pd-C in diethylether at very low hydrogen gas pressure. The resultant product **4** on basic hydrolysis furnishes 5. The procedure of Benn and Dodson (J. Org. Chem. **29**, 1142 (1964)] was followed for the preparation of Gugulsterone (9). The reduction of 16-DPA (**1**) with lithium aluminium hydride produces diol **6** which after Sigmatropic rearrangement in presence of p-toluenesulphonic acids acetic acid and acetic anhydride produces the diacetate **7**. Basic hydrolysis of the diacetate 7 followed by Oppenauer oxidation furnishes an 80 : 20 mixture of E&Z- Gugulsterone

(9).

5. BIOLOGICAL ACTIVITY

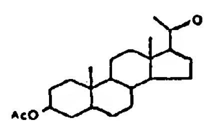
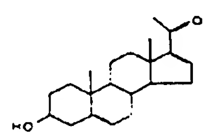
5.1 Hypolipidemic Activity

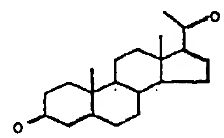
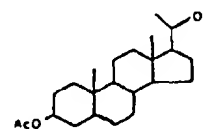
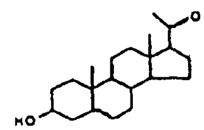
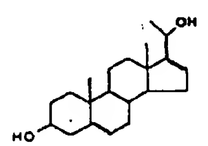
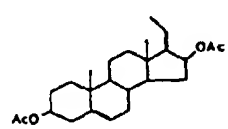
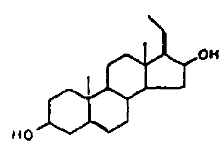
[0032] The primary hypolipidemic effect of these compounds were established in triton induced hyperlipidemia in Charles Foster rats. The compounds which exhibited significant lipid lowering effect in this model were then evaluated for their hypolipidemic effect in normal, and diet induced hyperlipidemic rats, rabbits and rhesus monkeys.

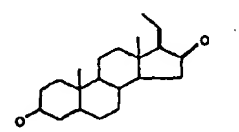
5.1.1. Hypolipidemic Activity In Triton Treated Rats

[0033] The cholesterol lowering effect of some representative compounds of pregrenadienols and - pregrenadienones as compared to clofibrate and gugulsterone in triton treated Charles Foster rats is described in Table-2

Table-2 : Cholesterol lowering effect of pregnane compounds as compared to Clofibrate in Triton treated rats

Compd. No.	Compound	Structure	Dose (i.p.) mg/kg	% Change S. Chol.
1	3ß-Acetoxypregna-5,16-dien-20-one (16-DPA)		50	-43
2	3ß-Hydroxypregna-5,16-dien-20-one		50	-46

3	4,16-Dienpregna-3,20-dione		50	-13
4	3ß-Acetoxypregna-5-en-20-one		100	-12
5	3ß-Hydroxypregna-5-en-20-one		100	-09
6	5,16-Dien-pregnane-3,20-diol		50	-10
7	5,17(20)-Dienpregna-3,16-diol-diacetate		50	-33
8	5,17(20)-Dienpregna-3,16-diol		50	-31

9	Gugulsterone		50	-44
10	Clofibrate		200	-15

[0034] The results showed that of the compounds tested, the highest effect was exhibited by 16-DPA (1) and its 3-des-acetyl analog **2** comparable to gugulsterone (**9**), and that the removal of double bond in ring D of **1** or **2** almost abolished the effect.

5.1.2 Hypolipidemic Activity of 3β-Hydroxypregna-5,16-dien-20-one (2) in Normal Rats

[0035] In normal rats, 3β-hydroxypregna-5,16-dien-20-one (2), at 50 mg/kg produced a significant lowering of serum cholesterol and triglycerides as describe in Table-3 below. The animals did not develop any tolerance to the compound even after administering for 30 days.

Table 3.

Hypolipidemic activity of 3β-Hydroxypregna-5,16-dien-20-one (2) in normal rats					
Treatment	**Serum Cholesterol (mg%)**		**%Fall**	**Serum Triglycrides (mg%) 30 Days**	**% Fall compared to control**
	0 Days	**30 Days**			
2(50mg/kg) (8)	71.5±1.8	43.2±2.9	40 40	42.0±2.8	35
Clofibrate (50 mg/kg) (6)	82.3±3.3	53.2±2.0	36	47.3±3.2	28
Normal saline (control) (6)	83.3±3.1	80.1±1.4	-	65.2±2.9	-
Mean values ± SD. Figure in parenthesis represent number of animals.					

5.1.3 Hypolipidemic Activity in Diet Induced Hyperlipidemic Rats

[0036] Twenty three normal male rats average weight 110-120 g were taken for study and were divided into four groups.Group I: animals recived special diet and 3β-hydroxypregna-5, 16-dien-20-one (**2**) 50 mg/kg p.o. in 1% gum acacia. Group II: animals received 3β-hydroxypregna-5, 16-dien-20-one (2), 100 mg/kg p.o. in 1% gum acacia and special diet. Group III:animals received special fat diet and 1% gum acacia and served as control. Group IV: animals were fed with stock diet and served as normal control. All animals were sacrificed at tile end of 36 days. Blood was drawn from the tail at 10 days and from the aorta at the time of sacrifice for estimation of serum cholesterol, triglycerides and HDL-cholesterol. LDL-cholesterol was calculated as described.
[Roschlau.P. In: Methods of Enzymatic Analysis 4th ed., H.U. Bergmeyer. Ed (Academic Press. New York) 1975 p 1890; Wahlefield, W.A. In: Methods of Enzymatic Analysis, 4th ed.; H.U. Bergmeyer, Ed.(Academic Press, New York) 1974 p 1831.]

Results

[0037] Animals treated with 3β-hydroxypregna-5, 16-dien-20-one (**2**), at 50 and 100 mg/kg showed a significant lowering in serum cholesterol by 31 and 59%, triglycerides by 55 and 62%. LDL-cholesterol by 27 and 74% respectively (Table - 4 & 5).

Table-4.

Effect of 3β-Hydroxypregna-5,16-dien-20-one (2) on serum cholesterol and triglycerides in hyperlipidemic rats					
Treatment	**Serum Cholesterol (mg%) Days**			**Serum Triglycerides (mg%)**	
	0	**10**	**36**	**0**	**36**
I **2**(50mg/kg)+HFD	69.1±9.9 (7)	212.4±23.8 (7)	165±26.7 (7)	48.8±7.0 (6)	61.5±10.5 (6)
%Decrease (Compound to group III)		**16**	**31**		**55**
II **2**(100mg/kg)+HFD	60.8±10.3 (6)	145.5±10,6 (6)	106.4±4.6 (5)	50±7.1 (5)	53.0±7.5 (5)
%Decrease (Compound to group III)		**48**	**59**		**62**
III HFD	73.2±5.8 (5)	325±29.8 (5)	293.2±16.6 (5)	48.75±5.1 (4)	137.5±8.5 (4)
IV Normal Diet		72±7.1 (5)			48.2±4.3 (5)
HFD= High fat diet, Values are Mean ± SD. Figures in parenthesis are number of animals					

Table-5.

Effect of 3β-Hydroxypregna-5,16-dien-20-one (2) on HDL and LDL-cholesterol in hyperlipidemic rats				
Treatment	**HDL-Cholesterol (mg/%)**		**LDL-Cholesterol (mg/%)**	
	Day 0	**Day 36**	**Day 0**	**Day 36**
I HFD	37.25±5.0	39.75±2.8 (4)	27.5±6.6 (4)	189.25±18.0 (4)
II **2**(50 mg/kg)+HFD	34.8±5.8 (7)	37.57±2.8 (7)	24.5±14.2 (7)	123.87±14.0 (6)
% Change		**181**		**271**
III **2**(100 mg/kg)+HFD	43.4±8.3 (5)	47.2±3.7 (5)	10.02±8.5 (5)	48.64+5.1 (5)
% Change		**161**		**741**
HFD = High fat diet, Values are Mean ± SD. Figures in parenthesis are number of animals				

5.1.4 Hypolipidemic Activity in Hyperlipidemic Rabbits

[0038] Effect of 3β-hydroxypregna-5,16-dien-20-one (**2**) was studied on hypercholesterolemic albino rabbits. Twelve male albino rabbits (approx 1.5-2 kg) on a stock diet were made hyperlipidemic by feeding daily cholesterol 0.5 g/kg in 2ml of groundnut oil for 45 days and then blood was drawn from the marginal vein in the ear of rabbits for serum cholesterol and triglycerides estimation.

[0039] Two controlled experiments were carried out for a period of three months. In one set of experiments, the control group received 0.5 g/kg of cholesterol for 90 days and 3β-hydroxypregna-5,16-dien-20-one (**2**) in dose of 100 mg/kg and 50 mg/kg while in the control group (given only cholesterol) a massive rise of serum cholesterol and triglycerides were seen after 90 days, the addition of 3β-hydroxypregna-5,16-dien-20-one (**2**) at 50 and 100mg/kg doses kept the rise well under control.The percentage decrease at 100mg dose was 28% at 30 days to 52% at 90 days for cholesterol and 45 to 81% for triglycerides. In the 50mg/kg dose group the decrease percentage ranged from 40% at 30 days to 50% at 90 days for cholesterol and 45% at 30 days to 75% at 90 days for triglyceride (Table-6)

Table-6.

Effect of 3β-Hydroxypregna-5,16-dien-20-one (2) in hyperlipidemic male-albino rabbits						
Treatment	**S. Cholesterol (mg%) Days**			**S.Triglycerides (mg%) Days**		
	30	**60**	**90**	**30**	**60**	**90**
Expt. I.A Control +cholesterol(0.5g/kg)	296.3	435.5	1337.5	69.0	150.0	161.0
Expt. I.B 2(100mg/kg)+cholesterol(0.5g/kg)	213.3	309.3	633.7	79.0	54.0	44.2
% Decrease	**28**	**42**	**53**	**46**	**64**	**81**
Expt. II.A Control +cholestrol(0.5g/kg)	317.8	531.8	1334.6	145.0	185.8	232.0
Expt. II.B 2 (50 mg/kg)+cholesterol(0.5g/kg)	190.5	305.5	660.3	79.5	67.0	56.5
% Decrease	**40**	**43**	**51**	**45**	**64**	**76**
Mean absolute Values mg/dl						

Table-7.

Hypolipidemic effect in hyperlipidemic rabbits (120 days experiment)				
Parameters	**Stock diet+ Normal saline**	**HFD**	**HFD+Guglip +2 (100mg/kg)**	**HFD + 2 (100mg/kg)**
Serum Cholesterol (mg/dl)	56.3±3.1	1367.0±203	258.6±28.2	350.0±28.1
% Decrease (Compared with HFD)			**81**	**74**
Serum Triglycerides	50.0±2.1	188.0±10.2	66.2±4.1	74.2±10.3
% Decrease (Compared with HFD)			**65**	**61**

5.1.5 Hypolipidemic Activity in Rhesus Monkeys

[0040] In Rhesus monkeys: 3β-Hydroxypregna-5,16-dien-20-one (**2**) was administered orally daily for 90 days in doses of 62.5, 125 or 650 mg/kg to different group of animals. Significant decrease in serum cholesterol was observed (45-52%). At 90 days, percentage decrease in triglycerides varied from 14 to 36%. Compound **2** caused a marked decrease in low density lipoprotein (75-90%) whereas changes in HDL-cholesterol were not significant (Table-8 & 9)

Table-8.

Effect of 3β-Hydroxypregna-5,16-dien-20-one (**2**) in Rhesus monkeys						
Treatment	**S. Cholesterol Days**			**S. Triglycerides Days**		
	0	**90**	**%Fall**	**0**	**60**	**% Fall**
Expt. I Control +1% gum acacia	119.8±16.6	90.5±9.0	26	82.0±2.5	62.0±1.0	24
Expt. II **2**(62.5 mg/kg)	156.8±23.0	83.3±8.5	47	77.5±7.0	66.3±6.9	14
Expt. III **2**(125 mg/kg)	133.3±8.5	65.8±2.2	50	69.8±9.9	60.0±2.9	13
Expt. IV **2**(650 mg/kg)	132.8±6.4	63.3±2.3	53	90.5±3.9	58.5±3.8	36
Mean ± SD values mg/dl. Each set of experiment involved 4 monkeys						

Table-9.

Effect of β-Hydroxypregna-5,16-dien-20-one (**2**) on HDL- and LDL-cholesterol in Rhesus monkeys						
Treatment	**HDL-Cholesterol Days**			**LDL-Cholesterol LDL-Cholesterol**		
	0	**90**	**% Change**	**0**	**90 %**	**Fall**
Expt. I Control +1% gum acacia	45.5±7.4	43.0±0.5	-6	57.9±16.2	35.0±8.8	39
Expt. II 2(62.5 mg/kg)	50.0±4.5	45.±2.5	-8	91.8±27.0	24.3±8.0	75
Expt. III 2(125 mg/kg)	46.3±2.0	50.8±0.5	+10	63.5±7.4	4.6±0.9	90
Expt. IV 2(650 mg/kg)	39.3±1.3	41.8±0.9	+6	72.7±8.4	13.1±0.7	82
Mean ± SD values mg/dl. Each set of experiment involved 4 monkeys.						

5.2 Hypoglycemic Activity

[0041] The compounds were tested for their hypoglycemic effects in normal, glucose loaded and streptozotocin induced diabetic rats. The experimental details of the testing of one such compound 3β-hydroxypregna-5,16-dien-20-one (**2**) is described below which possessed marked hypoglycemic effect in two models. (Tables 10 and 11)

5.2.1 Hypoglycemic Activity in Glucose Loaded Rats

[0042] The experiments were carried out with albino rats (Charles Foster strain) of either sex weighing 150-160 g. They were fed on laboratory diet prepared by M/s Lipton India Ltd. and maintained under 12 hr. light/dark cycle at 25±2°C.

[0043] The animals were divided into eight groups, each of six rats; group I was given 1% gum acacia (0.1ml/100g body weight) intragastrically (p.o.) and the group II were given 3β-hydroxypregna-5,16-dien-20-one (**2**) in 1% gum acacia, Animals of groups III were given the standard antidiabetic drug, tolbutamide in the similar fashion. 2.0g/kg glucose was given p.o. to all the rats along with the vehicle/Compound/Standard antidiabetic drug. Blood samples were taken from retroorbital plexus at periodic intervals. Glucose levels in the blood samples were measured by glucose oxidase method [Bergmeyer and Benut, 1963 cited in "Methods of Enzymatic Analysis" ed. H Bergmeyer, Verlag Chemie, GmBH, Weinheim, Beroster, pp123, Academic press, New York].

Table-10.

Effect of 3β-Hydroxypregna-5,16-dien-20-one (**2**) and standard antidiabetic drug on post-prandial blood glucose level after challenge with glucose						
Group	**Blood Glucose Level (mg/dl)**					**Maximum Blood Glucose Change (%)**
	0 min	**30 min**	**60 min**	**90 min**	**120 min**	
Control	40.35 ± 2.9	102.3 ±6.11	68.91 ±1.95	66.48 ±2.12	50.61 ± 1.27	
Compund **2** (100 mg/ kg) + Glucose	42.43 ± 1.64	76.15 ± 6.07 (48)	56.06 ± 4.65 (55)	55.06 ± 2.61 (54)	49.59 ± 1.66 (34)	-18.7
Tolbutamide (100 mg/kg) + Glucose	38.15 ± 2.02	76.31 ± 6.29 (35)	27.26 ± 3.1 (100)	24.64 ± 1.92 (100)	23.07 ± 3.11 (100)	-41.7
Figure in parenthesis indicates % inhibition compared to control Mean ± SD values mg/dl. Each set of experiment involved 6 rats,						

5.2.2 Hypoglycemic Activity in Streptozotocin Induced Hyperglycemic Rats

[0044] Hyperglycemia in rats was produced by streptozotocin treatment. The animals showing blood glucose levels between 250-350 mg/dl were selected. Blood samples were collected after treatment at intervals and blood glucose levels were estimated immediately. The results showed lowering in blood glucose level within 1 hr and the maximum

fall was observed at 12 hrs.

Table 11:

Effect of 3β-Hydroxypregna-5,16-dien-20-one (2) and Tolbutamide on blood glucose level of streptozotocin induced diabetic rats.										
Treatment	Blood glucose level mg/dl									
	0hr	1hr	2hr	3hr	4hr	5hr	6hr	7hr	8hr	24hr
I Streptozotocin (50 mg/kg i.p.)	301 ± 7	314 ± 11	318 ± 12	328 ± 10	328 ± 15	333 ± 21	347 ± 19	355 ± 24	340 ± 22	349 ± 12
II Streptozotocin (50 mg/kg p. o)*2 (100 mg/kg p. o.)	274 ± 9	243* ± 10 (11.3)	231* ± 14 (15.6)	203** ± 20 (25.9)	183*** ± 14 (33.2)	157*** ± 21 (42.7)	133*** ± 16 (51.4)	102*** ± 9 (62.7)	91*** ± 8 (66.7)	128*** ± 18 (52.2)
IV Streptozotocin (50 mg/kg i.p. + Tolbutanfide (100 mg/kg p. o.)	292 ± 10	218** ± 14 (25.3)	207** ± 17 (29.1)	202** ± 19 (30.8)	195** ± 23 (33.2)	183** ± 23 (37.3)	174*** ± 23 (40.4)	192** ± 29 (34.2)	242* ± 18 (17.1)	332 ± 26
Mean ± SD values mg/dl., Parenthesis shows % lowering from zero hour value, *P < 0.05, **P < 0.01, ***P < 0.001										

5.3. Antioxidant Activity:

[0045] The free radical oxidative stress has been implicated in the pathogenesis of a variety of human disease conditions including atherosclerosis. Polyunsaturated fatty acids within cell membranes and lipoproteins are oxidized and resulting active specie modify macrophages and bystander cells monocytes which then move to subendothelial space, engorge cholesteryl esters and are transformed into what are known as foam cells. Group of these foam cells form atherosclerotic plaques in the intima. The antioxidant potential of compound **2** was evaluated against metal induced oxidation of LDL as well as generation of hydroxy (OH) radical.

[0046] *In vivo* experiment with cholesterol fed animals caused marked formation of lipid peroxides in serum lipoproteins. Simultaneous treatment with compound **2** caused significant reversal of the lipid peroxide levels in serum VLDL, LDL and liver in cholesterol fed animals. However gemfibrozil failed to protect the phenomenon of lipid peroxidation (Table 12). Human serum LDL was oxidized with Cu^{+2} in different concentrations of test compound in 0.05 mole PBS, pH 7.4 for 16 hr at 37°C. Thiobarbituric acid reactive lipid peroxides are measured by standard procedure. Compound **2** and α-tocopherol inhibit the generation of LDL lipid peroxide in concentration dependent manner. However the gemfibrozil at tested concentrations did not inhibit the oxidative modification (Table 13). Cu^{+2} induced oxidation in LDL lipids are mainly due to the free OH radical during incubation. Therefore the effect tested for generation of OH radical in vitro in nonenzymatic system of Fe^{+2}, sodium ascorhate, hydrogenperoxide and deoxyribose oxidative attack of oxy radical on deoxyribose caused fragmentation and formation of dialdeloyde which are spectrophotometrically measured after their reaction with thiobarbituric acid. As observed in case of oxidation of LDL by metal ion, the compound **2** inhibits the generation of OH radical in concentration dependent manner. However, the activity of **2** is of low order to that of mannitol, a selective inhibitor of OH radical (Table 14)

Table 12:

Effect of 3β-Hydroxypregna-5.16-dien-20-one (2) on lipid peroxidation in cholesterol fed hyperlipidemic rats				
			Cholesterol + Drug (25 mg/kg)	
Serum / Tissue	Control	Cholesterol Fed	Compound 2	Gemfibrozil
Serum a	675 ± 82	1228 ± 130 (+82)	931.5 ± 51 (-24)	1161 ± 74 (-5)

Table 12: (continued)

Effect of 3β-Hydroxypregna-5.16-dien-20-one (**2**) on lipid peroxidation in cholesterol fed hyperlipidemic rats				
			Cholesterol + Drug (25 mg/kg)	
Serum / Tissue	Control	Cholesterol Fed	Compound 2	Gemfibrozil
VLDL a	237 ± 25	379 ± 20 (+60)	310 ± 15 (-18)	346 ± 10 (-9)
LDL a	351 ± 17	604 ± 24 (+72)	421 ± 20 (-30)	568 ± 62 (-6)
HDL a	123 ± 13	138 ± 17 (+12)	125 ± 10 (-9)	134 ± 13 (-3)
Liver b	74 ± 8	274 ± 28 (+57)	212 ± 16 (-23)	253 ± 26 (-8)
Values are mean ±SD of 6 rats; a=n mol MDA/dl, b=n mol MDA/g. Values in the parenthesis below drug treated groups are % reversal as compared with cholesterol fed rats.				

Table 13:

Effect of 3β-Hydroxy-pregna-5,16-dien-20-one (**2**), gemfibrozil and α-Tocopherol on low density lipoprotein oxidation.						
Concentration		Compound 2	Gemfibrozil	α-Tocopherol		
(μmol/ml)		MDA	MDA	Conc. (μmol/ml)		MDA
None		56.5 ± 3	52.0 ± 5.4	None		47.4 ± 3.8
2.5	Ref.	56.0 ± 5.8	52.8 ± 5.7	0.25	Ref.	47.4 ± 4.5
	Exp.	41.4 ± 1.7 (26)	53.0 ± 6.0 (-)		Exp.	44.7 ± 4.5 (3)
5.0	Ref.	56.4 ± 5.6	50.7 ± 6.1	0.5	Ref. Exp.	47.3 ± 4,5
	Exp.	28.7 ± 4.0 (49)	48.8 ± 6.7 (5)			41.6 ± 4.0 (13)
10.0	Ref.	40.6 ± 3.7	49.4 ± 5.2	1.0	Ref.	47.4 ± 3.9
	Exp.	10.6 ± 0.5 (74)	43.8 ± 4 9		Exp.	35.0 ± 3.8
20	Ref.	37.2 ± 0.8	49.0 ± 2.9	2.0	Ref.	47.4 ± 4.4
	Exp.	4.0 ± 0.5 (89)	41.4 ± 5.0 (16)		Exp.	24.3 ± 2.0 (51)
Values expressed as n mol MDA/mg protein are mean ±SD of four separate experiments. Values in the parenthesis are % inhibition.						

Table 14 :

Inhibition of Hydroxy (OH) radical formation in nonenzymatic system.				
Concentration	Compound 2	Gemfibrozil	α-Tocopherol	
(μmol/ml)	(nmol MDA/hr)	(nmol MDA/hr)	Conc. (μmol/ml)	(nmol MDA/hr)
None	90.4 ± 3.8	83.4 ± 7.5	None	78.2 ± 7.0
5	65.0 ± 5.4 (28)	79.9 ± 6.8 (4)	1	60.4 ± 7.0 (23)

Table 14 : (continued)

Inhibition of Hydroxy (OH) radical formation in nonenzymatic system.				
Concentration	Compound 2	Gemfibrozil	α-Tocopherol	
(μmol/ml)	(nmol MDA/hr)	(nmol MDA/hr)	Conc. (μmol/ml)	(nmol MDA/hr)
10	52.8 ± 4.2 (42)	78.5 ± 8.0 (5)	2	45.2 ± 3.3 (42)
20	23.3 ± 1.8 (74)	76.8 ± 7.4 (8)	3	32.4 ± 3.5 (59)
30	11.7 ± 1.3 (87)	76.4 ± 3.8 (8)	4	27.1 ± 2.8 (65)
40	10.4 ± 2.0 (88)	74.3 ± 6.3 (11)	5	20.9 ± 2.0 (73)
50	9.9 ± 0.8 (90)	72.4 ± 8.0 (13)	-	-
Values are mean ± SD of four separate observations. Values in the parenthesis are % inhibition.				

5.4. Cardiac Protection:

[0047] The underlying cause of myocardial infarction is believed to be the progressive deposition of lipids and fibrotic material into the arterial wall. These pregnadienols and pregnadienous also provided cardiac protection as assessed in isoproterneol induced myocardial necrosis in rat model, which produces myocardial infarction due to an increased blood pressure and heart rate. The protection was comparable with that of gemfibrozil as described in Table 15.

Table 15 :

Effect of 3β-Hydroxyregna-5,16-dien-20-one(2) on serum and tissue parameters of heart necrosis at 25 mg/kg (p. o.) in rats.			
Parameters	isoproterneol (85 mg/kg, p.o.) % Change	Isoproterneol + Drug (25 mg/kg) % Protection	
		Gemfibrozil	Compound 2
Serum			
CPK	109.9 I	33.9	35.7
GOT	41.6 I	24.9	27.0
GPT	85.0 I	33.4	22.7
Alkaline Phosphatase	28.8 I	41.2	24.0
Heart			
Ca. - ATPase	44.5 I	30.2	30.2
Glycogen	20.2 I	32.1	25.1
Lipid peroxide	65.8 I	69.8	72.6
Phospholipase	216.0 I	28.2	74.2

5.5. ANDROGENIC ACTIVITY

[0048] The relative affinity of a few selected compounds in the series for cytoplasmic androgenic receptors present in human breast tumour cells MCF-7 (Michigan Cancer Foundation, MCF, USA) was estimated and compared with 4,5-dihydrotestosterone (DHT). The results showed that the compounds 2,3 and 5 have no or only negligible binding affinity which therefore would be a reflection of their low androgenic effect.

5.6. PROGESTATIONAl AND ANTIPROGESTATIONAL ACTIVITY

[0049] The relative affinity of compounds for cytoplasmic progesterone receptors present in human breast tumour cells (MCF-7) were estimated and compared with 16-ethyl-21-hydroxy-19-norpregna-4-ene-3,20-dione (Org 2058). The experiments conducted revealed that the compounds 2,3 and 5 have no or only negligible binding affinity.

[0050] The progestational activity was also tested *in vivo* by Clauberg assay method. The degree of endometrial proliferation was estimated on the McPhail scale where 3' or 4' was considered as a full progestational effect, 3β-Hydroxypregna-5,16-dien-20-one (**2**) did not exhibit any activity even at 200 mg/kg dose, whereas progesterone showed, as expected marked progestational activity even at 50 mg/kg.

Claims

1. Use of a compound represented by the structural formula (I) as shown in the accompanying drawings, containing at least one olefinic bond in or on their D-ring in the manufacture of a medicament for the treatment or prophylaxis of a hypolipidemic and/or hypoglycemic condition.

2. Use of a compound represented by the structural formula (I) as shown in the accompanying drawings, containing at least one olefinic bond in or on their D-ring in the manufacture of a medicament for the treatment or prophylaxis of a diabetic condition.

3. Use of a compound represented by the structural formula (I) as shown in the accompanying drawings, containing at least one olefinic bond in or on their D-ring in the manufacture of a medicament for lowering serum cholesterol, triglyceride and/or glucose levels in a subject with obesity or a diabetic condition.

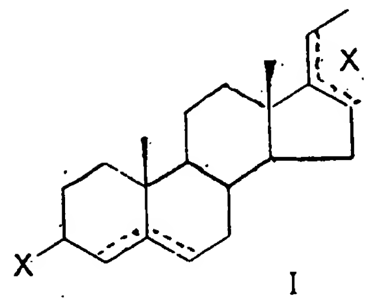
4. Use as claimed in any of claims 1 to 3, wherein the compound of formula (I) is characterised as a pregnadienone or pregnadienol.

5. Use as claimed in any of claims 1 to 4, wherein the compound of formula (I) is 3β-hydroxy-pregna-5,16-dien-20-one, which is represented by the structural formula (II) as shown in the accompanying drawings.

6. Use as claimed in any of claims 1 to 5, wherein a compound of formula (I) is optionally administered as an admixture with one or more other therapeutic agents selected from anti-platelet, anti-artherosclerotic, hypolipoproteinic and antidiabetic drugs.

7. Use as claimed in any of claims 1 to 5 wherein the medicament is in the form of a tablet, capsule or injectible.

8. A compound of formula (I) for use in the treatment of a hypolipidemic and/or hypoglycemic condition.



I

Figure 1 (I)

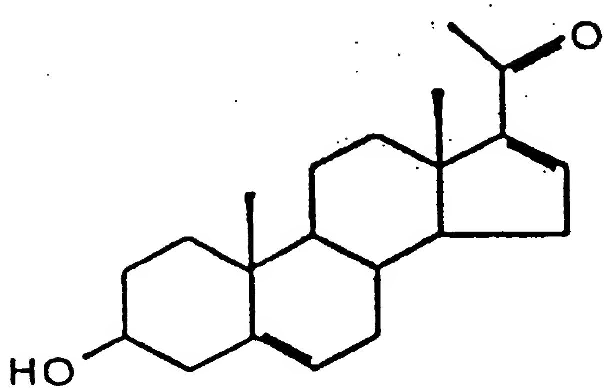
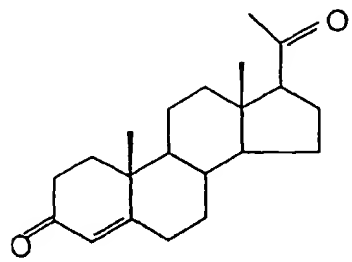
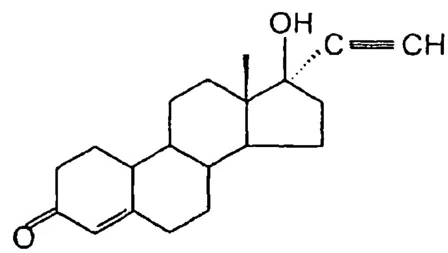


Figure 1 (II)



PROGESTERONE



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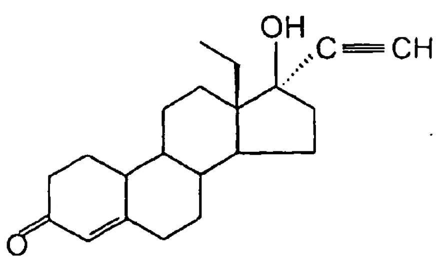
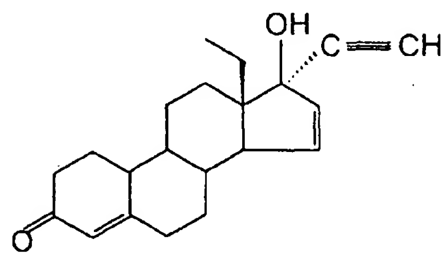


LEVONORGESTREL



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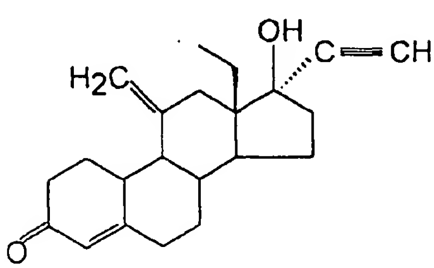
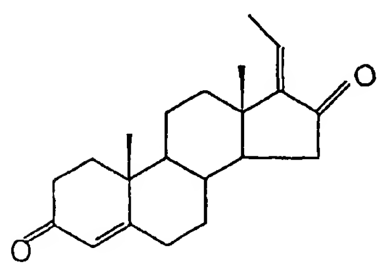


3-KETODESOGESTREL



GUGULSTERONE

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 99 30 2556

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	11 October 1999	Gac, G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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EPO FORM 1503 03.82 (P04C01)

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Application Number
EP 99 30 2556

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Application Number
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The members are as contained in the European Patent Office EDP file on
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11-10-1999

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US 2752370	A	26-06-1956	NONE	
US 3475464	A	28-10-1969	NONE	
DE 1301999	B		NONE	

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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Myrrh

Back

SYNONYMS
African Myrrh, Arabian Myrrh, Balsamodendron Myrrha, Commiphora Resin, Guggal Gum, Guggal Resin, Gum Myrrh, Heerabol Myrrh, Mo Yao, Myrrha, Somali Myrrh, Somalian Myrrh, Yemen Myrrh

[top]

FAMILY
Burseraceae

[top]

GENUS SPECIES
African Myrrh, Somali Myrrh: *Commiphora molmol* Engler, *Commiphora myrrha*, Arabian Myrrh, Yemen Myrrh: *Commiphora madagascariensis* (syn. *Commiphora abyssinica*), *Commiphora erythraea*, et al.

[top]

TYPE
Shrub or small tree

[top]

PART USED
Oleo-gum resin from stems

[top]

LOCATION
Abyssinia, Erythrea, North Africa, Somalia, southern Arabia, Sudan, Yemen

[top]

ACTIONS
Alterative, anodyne [3,4], anticatarrhal, antidiabetic, antifungal, antihypercholesterolemic, antihyperglycemic, anti-inflammatory, antimicrobial [2], antipyretic, antiseptic, antitussive, astringent [3,4], bitter, bowel cleanser, carminative, circulatory stimulant, emmenagogue, expectorant, fragrance, immunostimulant (increases WBC count)[2], protective, stimulant, stomach cleanser, stomachic, tonic, uterine stimulant, vulnerary

[top]

COMMISSION E ACTIONS
Astringent

[top]

INDICATIONS
Abrasions, amenorrhea, arthritis, asthma, athlete's foot, bedsore, bronchitis, bronchorrhea, brucellosis, cancer, canker sores, chronic cough, common cold, conjunctivitis (eyewash), cystitis, cystorrhea, dermatitis, diabetes mellitus, endometritis, furunculosis, gastroenteritis, gingivitis [1], halitosis [1], hemorrhoids, herpes simplex, hoarseness, hypercholesterolemia, hyperglycemia, hysteria, lochia, menoxenia, mouth ulcers, muscle spasms, nephritis, periodontitis, pyorrhea, rheumatism, sinus problems, sinusitis, sore throat, stomatitis, thrush, tonsillitis, toothache [1], urethritis, uterine inflammation, vaginitis, wounds

[top]

COMMISSION E INDICATIONS
Topical: Inflammation of oral mucosa, Inflammation of pharyngeal mucosa

[top]

PREPARATION & DOSAGES
Commission E: Tincture: Dab on inflammed area 2-3x/day; Rinse or Gargle: 5-10 drops in a glass of water; Dental Powder: 10% resin
Decoction: 3-12 g, in 2-3 doses
Mouthwash or gargle: 5 ml of tincture in a glass of water (3x/day)
Tincture: 1:5, 90% ethanol, dose 1-2.5 ml (3x/day)

[top]

CONTRAINDICATIONS
Lactation, menorrhagia, pregnancy.

[top]

DRUG INTERACTIONS
May potentiate antidiabetic (hypoglycemic) drugs. Antidiabetics, hypoglycemics. Myrrh preparations may interfere with hypo- or hyperthyroid drugs.

[top]

SIDE EFFECTS
(Possible adverse effects and/or overdose effects) Convulsions, drowsiness, lethargy. Doses larger than 2-4 g may cause nephritis and diarrhea.

[top]

SAFETY
GRAS.

[top]

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myrrh. *Nature* 1996;376:29.

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L28 ANSWER 21 OF 122 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 7
AN 1999:632461 CAPLUS
DN 131:331981
TI The acetylcholinesterase inhibitor, ENA 713 (Exelon), attenuates the working memory impairment induced by scopolamine in an operant DNMTP task in rats
AU Ballard, Theresa M.; McAllister, Kevin H.
CS Nervous System Department, Novartis Pharma Inc., Basel, CH-4002, Switz.
SO Psychopharmacology (Berlin) (1999), 146(1), 10-18
CODEN: PSCHDL; ISSN: 0033-3158
PB Springer-Verlag
DT Journal
LA English
AB Rationale: The disruption of working **memory** in the delayed non-matching to position (DNMTP) task by the muscarinic antagonist, **scopolamine**, is considered to be a **model** of the spatial working **memory** deficit in **Alzheimer**'s disease (AD) patients. Objective: To investigate whether ENA 713 (Exelon) (0.1, 0.5 mg/kg, IP), an acetylcholinesterase inhibitor, would reverse the effects of **scopolamine** in the DNMTP task. Methods: Male Lister Hooded rats were trained to criterion in an operant DNMTP task (0- to 16-s delay intervals) before receiving vehicle, **scopolamine** (0.05 mg/kg, SC) alone, ENA 713 (0.1, 0.5 mg/kg, IP) alone, or combinations of **scopolamine** and ENA 713, in two variations of the task - with and without barriers inserted between the food magazine and the two levers. Barriers were inserted to prevent the use of positional strategies to perform the task, since this behavior may confound the conclusions of the effect of drugs on working **memory**. Results: It was found that: (i) **scopolamine** significantly reduced choice accuracy delay-dependently in both test situations while modifying non-mnemonic measures of task performance delay-independently, indicating an impairment of working **memory**; (ii) ENA 713 (0.5 mg/kg) significantly attenuated the **scopolamine**-induced impairment of working **memory** and significantly reduced the **scopolamine**-induced changes in some non-mnemonic measures of task performance; (iii) the presence of barriers did not alter the effects of **scopolamine** and ENA 713 on working **memory**. Conclusion: ENA 713 reversed the working **memory** deficit induced by **scopolamine**. These results are consistent with the attenuation of learning and **memory** disruptions due to cholinergic dysfunction by ENA 713 in other preclin. assays, and predict a drug-induced improvement in working **memory** in AD patients.

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ORGN . . .
(STY): FOLKLORE Classification (CC): ANTIHYPERGLYCEMIC ACTIVITY
Extract type: HOT H2O EXT
Dosage Information: ORAL; HUMAN ADULT
Comment(s): USED FOR **DIABETES**.
ORGN Class: DICOT Family: BURSERACEAE Genus: COMMIPHORA Species: WIGHTII
Synonym(s): BALSAMODENDRON WIGHTII
Common name(s): **GUGGUL**
Organism part: DRIED GUM
Geographic area (GT): INDIA; SAS
TYPE OF STUDY (STY): FOLKLORE Classification (CC): APHRODISIAC ACTIVITY
Extract. . .